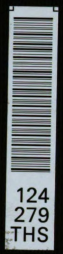

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ANAEROBIC CELLULOLYTIC ACTIVITY IN
THE LITTORAL SEDIMENTS OF A HYPEREUTROPHIC LAKE

presented by

Pamela L. Salvas

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of the requirements for

M.Sc. degree in Microbiology

Major professor

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ANAEROBIC CELLULOLYTIC ACTIVITY IN LITTORAL SEDIMENTS OF A HYPEREUTROPHIC LAKE

By

Pamela Lynn Salvas

A THESIS

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ABSTRACT

ANAEROBIC CELLULOLYTIC ACTIVITY IN LITTORAL SEDIMENTS
OF A HYPEREUTROPHIC LAKE

By

Pamela Lynn Salvas

Cellulolytic activity in littoral sediments of a hypereutrophic lake was assessed using natural plant material and cotton fiber, and the characteristics of the anaerobic cellulolytic microbiota were examined during the course of this study. Sediment parameters were monitored (H_2S, temperature, cellulose pool size) to determine environmental conditions. Cellulolytic anaerobic bacteria were isolated and characterized by standard methods. The activity of sediments and isolates on different quality cellulose substrates (carboxymethylcellulose, alpha-cellulose, and ground Nuphar--the yellow water lily) was determined.

Anaerobiosis in Wintergreen Lake littoral sediments is rarely disturbed and the localization of cellulolytic activity primarily in a zone below the sediment surface indicates cellulose metabolism is predominantly anaerobic. 84% of Nuphar biomass buried in the sediments is degraded during an annual cycle. Cellulolytic anaerobes isolated include members of the genera Eubacterium, Cellulomonas, Clostridium, and Propionibacterium. The four most commonly isolated organisms showed an ability to hydrolyze ground Nuphar which provides evidence for their important role in the metabolism of natural cellulosic inputs to the littoral sediments. A factor clearly influencing cellulose degradation by littoral sediment microbiota is the quality of the cellulose substrate. Sediment temperature also influences the rate of cellulose decomposition.

To My Good Friends

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INTRODUCTION

Macrophyte production in small, shallow lakes may contribute up to 73% of the lake's total annual primary productivity (Wetzel, 1975). Grazing by herbivores is of minimal significance in most macrophyte communities (Fenchel, 1970; Mann, 1972; Westlake, 1965). Utilization of macrophyte productivity is therefore through detrital-based food webs after plant senescence (Westlake, 1965; Harrison and Mann, 1975; Fenchel, 1977; Mann, 1972). Cellulose is the major structural carbohydrate in higher plants (Anthony, et al., 1969; Sculthorpe, 1967) and comprises a large portion of this plant detritus. Most decomposition of this cellulosic material is displaced to the sediments because its particulate nature causes sinking (Brock, 1966; Wetzel, 1975).

The type of sediment microbial metabolism is primarily determined by oxygen availability in the sediments (Hayes, 1964). Even in highly aerated systems oxygen diffusion through sediments occurs very slowly and biological oxygen demand from microbial activity at the sediment-water interface severely restricts oxygen penetration into the sediments (Brock, 1966). Sediments which are organically enriched become anaerobic due to the intensity of microbial decomposition and slow diffusion of oxygen (Stumm and Morgan, 1969).

Rapid cellulose decomposition occurs in anaerobic habitats when environmental constancy allows the evolution of a stable anaerobic cellulolytic microbial community. Examples of such habitats are the rumen (Hungate, 1975), and anaerobic sludge digester systems (Toerien et al., 1967). In both of these systems, anaerobiosis is strictly maintained, and substrate is continuously available. Conceptually, littoral sediment systems sustaining high levels of organic matter are similar to these

systems in their anaerobic nature and constant availability of cellulosic substrates. This suggests that anaerobic cellulolytic population will develop in such sediments and cellulose degradation will proceed actively.

Although oxygen plays the most important role in determining the type of sediment cellulose metabolism, temperature may exert a strong effect on the rate and extent of that activity in the sediments. Lake sediments, particularly in shallow littoral zone areas, display a large seasonal temperature fluctuation. Oppenheimer (1960) found that decomposition slowed dramatically but did not cease when winter temperatures decreased to $4^{o}C$ in shallow marine bay sediments. Godshalk (1977) obtained similar results in laboratory experiments by measuring aquatic plant decomposition at summer ($25^{o}C$) and winter ($10^{o}C$) temperatures. Littoral zone sediments of heavily vegetated lakes receive significantly large portions of their annual organic matter input during the fall, as seasonal temperature begins to decline. The effect of the combined factors on sediment metabolism is not known.

The qualitative nature of the cellulosic inputs to the sediments also bears on the rate and extent of decomposition. The fibrous structural components of aquatic plants are complexes of cellulose in association with lignin, hemicellulose and other substances (Boyd and Goodyear, 1971; Wetzel, 1975; Godshalk, 1977). Due to the nature of enzymic hydrolysis of cellulose, the degradation of such complex compounds depends on the accessibility of the cellulose to the hydrolytic enzymes (Cowling, 1975).

The object of this study was to examine cellulose degradation in the littoral sediments of a hypereutrophic lake, and to determine the characteristics of the anaerobic cellulolytic community. Testing of

the following hypotheses formed the structure around which the work was based:

1. Because of organic enrichment and slow diffusion of oxygen into littoral sediments, the sediment environment will be primarily anaerobic in nature.

2. Because cellulosic input is high and anaerobiosis predominates, there will be an active anaerobic cellulolytic community in the littoral sediments.

3. The rate and extent of cellulolytic activity by littoral sediment microbiota will be influenced by the quality of the substrate and seasonal fluctuations in sediment temperature.

METHODS AND MATERIALS

The Study Site

Wintergreen Lake, a small hypereutrophic lake located in the W. K. Kellogg Bird Sanctuary (Kalamazoo County, Michigan), was the site of the study (Molongoski, 1976). The lake has extensive macrophyte stands in the littoral zone (Figure 1), in which floating-leafed plants, especially the yellow water lily (Nuphar advena), predominate in biomass (Table 1). Significant amounts of cellulose from these plants are introduced into the sediments of Wintergreen Lake annually; the greatest inputs occur during the fall senescence of the macrophyte community. The study was conducted in the sediments of a dense Nuphar bed on the western side of the lake. Organic content of the sediments in this region ranges from 39-51% annually (P. L. Salvas, unpublished data).

Field Studies

Sediment Parameters

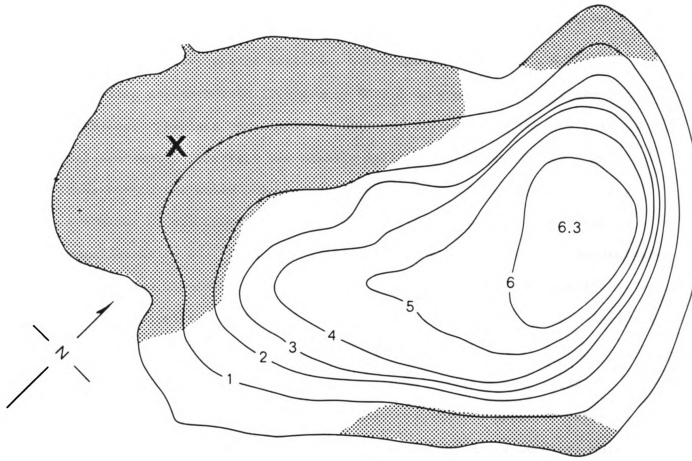
Sediment interstitial water samples were collected at intervals during the field study using pore water samplers similar to those described by Hesslein (1976) and by Winfrey and Zeikus (1977). Sampling ports were

Figure 1. Bathymetric map of Wintergreen Lake showing the extent of macrophyte cover (shaded areas). The study site is marked with an X. (Map modified from Manny, 1972).

WINTERGREEN LAKE

KALAMAZOO COUNTY, MICHIGAN

R.9W.,T.1N. Sec.8



ELEVATION 271m, AREA 15.8 ha

0 50 100 150
METERS

CONTOUR INTERVALS IN METERS

Table 1. Estimated contributions to macrophyte beds in Wintergreen Lake by emergent, floating-leafed, and submergent aquatic plants.

	Percent of total biomass	Predominant species (in order of dominance
Floating-leafed plants	60	Nuphar advena Nymphaea tuberosa
Submergent plants	30	Ceratophyllum demersum Potemogeton spp.
Emergent plants	10	Decodon verticillatus Polygonum spp. Typha sp. Scirpus sp

filled with deoxygenated distilled water and covered with a 0.22 μm pore size polycarbonate (Nucleopore Corp.) or acetonitrile (Gelman Corp.) membrane. Samplers were inserted into the sediments by hand so that the sediment-water interface was clearly defined and approximately 5 cm remained in the water column. Equilibration time for each sampler was two weeks. Upon retrieval of the sampler, water samples were taken immediately in the field for sulfide determinations using 7 ml vacutainers. One ml of 0.2% zinc acetate was added to each vacutainer in the field to fix the sulfide as its zinc salt (Caldwell and Tiedje, 1975). Analysis of sulfide was by the method of Cline (1969).

Cellulose pool size in Wintergreen Lake littoral zone surface sediments was determined from duplicate core samples taken biweekly or monthly throughout the study period. Surface sediments (0-6 cm) were dried at 50°C and finely ground. Organic content was determined by weight loss after combustion at 550°C for 18 hours. Sediment samples were prepared for cellulose analysis by extracting non-cellulosic carbohydrates with acetic-nitric reagent (Updegraff, 1969) and then assaying samples for carbohydrate by the modified phenol-sulfuric method (Gerchakov and Hatcher, 1972; Liu et al., 1973).

Temperature in the surface sediments of the study site was measured with a YSI telethermometer.

Litterbag Study

Standing Nuphar leaves and petioles were collected in mid-September, 1977 during the fall senescence period. The leaves and petioles were separated and dried at 60°C. Fiberglas litterbags (15 x 15 cm, 1.5 mm mesh size) were filled with 6 g or either Nuphar leaves or petioles. Four leaf-containing and four petiole-containing litterbags were tied to a marker stake; stakes and litterbags were distributed throughout the Nuphar bed on the west end of Wintergreen Lake (Figure 1). Each litterbag was buried in the surface (2-6 cm) sediments surrounding its marker stake. Water depth ranged from 0.5 to 1.0 m.

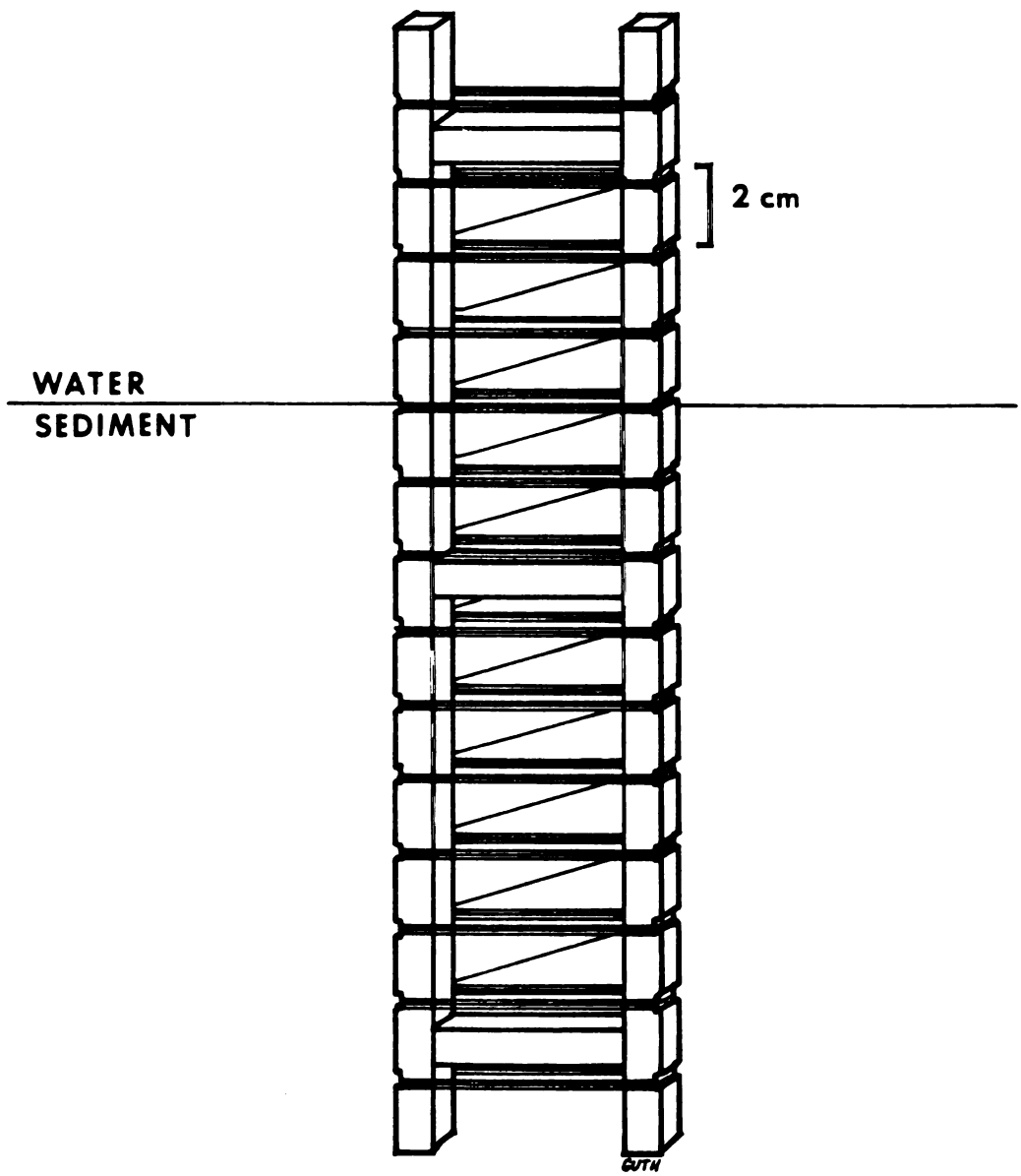
Litterbag sets were retrieved periodically over a one year period. Upon retrieval, litterbags were wrapped in plastic to reduce exposure to air and transported immediately to the laboratory. From one bag each of petiole and leaf material, approximately 15 sections (10-15 mm in diameter) of decomposing plant tissue were chosen randomly and fixed in 2% glutaraldehyde in phosphate buffer for one hour. Fixed samples were successively dehydrated in 15, 40, 70 and 100% ethanol. Subsequently, 3 to 5 random samples from each sampling date were dried in a Sorvall model 49300 carbon dioxide critical point dryer, mounted on stubs, and vacuum coated with gold before viewing with a scanning electron microscope.

The remaining litterbags were rinsed with tap water. Plant material was carefully picked out, dried at 50°C and weighed to determine weight loss. Cellulose content of the residual plant material was analyzed by the fiber extraction method of Goering and Van Soest (1970).

Cotton Fiber Degradation

The first sign of degradation of fibrous cellulose is a loss in tensile strength (Halliwell, 1963). In order to obtain the relative cellulolytic activity with depth in Wintergreen littoral sediments, loss of tensile strength of cotton fibers incubated in the sediments was assessed. Seventy-five cm lengths of #60 mercerized cotton thread were evenly wound about notched plexiglas frames (30 cm x 6 cm) at 2 cm intervals (Figure 2). Triplicate samplers were inserted into the sediments of Wintergreen Lake littoral zone so that approximately 10 cm extended into the water column and 20 cm was buried in the mud. Samplers were incubated *in situ* for one week on two separate occasions. To remove the samplers, plexiglas cores (7.5 cm diameter) were carefully placed around each frame and both sediment and sampler were removed, keeping the sediment-water interface intact. Cores were stoppered and transported to the laboratory. In the laboratory, cores were continuously flushed with oxygen-free nitrogen gas during handling. The incubation depth relative to the sediment surface of each fiber interval was recorded by measuring the position of the sampler with respect to the sediment-water interface. At each depth interval fibers were removed, rinsed with distilled water and dried at 50°C for tensile strength loss measurements. The relative loss in tensile strength of sediment incubated cotton fibers was measured by comparing the weight needed to break the field incubated cotton fiber relative to that needed to break unincubated control fibers. Subsamples of the incubated fiber from 0, 6, and 12 cm were prepared for scanning electron microscopy and viewed as described for the litterbag study.

Figure 2. Plexiglas frame wound with cotton fiber used in Wintergreen Lake littoral sediments to determine cellulolytic activity with depth.


2 cm
WATER
SEDIMENT
GUTH

Anaerobic Cellulolytic Bacterial Numbers in Wintergreen Lake Littoral Sediments

Enumeration of anaerobic cellulolytic microorganisms in littoral sediments was determined seasonally during 1977-1978 by the Most Probable Number (MPN) technique. Media composition of MPN tubes was as follows: (per liter), K_2HPO_4, 0.45 g; KH_2PO_4, 0.45 g; $(NH_4)_2SO_4$, 0.90 g; $MgSO_4$, 0.09 g; $CaCl_2$, 0.09 g; distilled H_2O, 865 ml; 10% $NaHCO_4$, 50 ml; 2% ball-milled Whatman Number One filter paper (aqueous suspension), 75 ml; 1% rezazurin, 2 ml; cysteine HCl hydrate, 0.5 g; casamino acids, 0.10 g; final pH before autoclaving was adjusted to 7.2. Mineral salts used for serial dilutions were the same but lacked cellulose and casamino acids.

Sediment cores from the study site were obtained using plexiglas cores (7.5 cm diameter). Cores were stoppered and transported to the laboratory without disturbing the sediment-water interface. The overlying water was siphoned off and surface sediments (0-6 cm) were transferred from the cores to 250 ml Erlenmeyer flasks while flushing with oxygen-free nitrogen gas. The flasks were stoppered and introduced into an anaerobic chamber (Coy Manufacturing, Ann Arbor, Mi.) containing 85% nitrogen, 10% hydrogen, and 5% carbon dioxide atmosphere. Analyses were made on 2-3 cores. The sediments were mixed well by magnetic stirrer, ten-fold serial dilutions were prepared in prereduced mineral salts, and 0.5 ml from each dilution was inoculated into five tubes containing 4.5 ml of MPN media. The tubes were tightly stoppered and removed from the anaerobic chamber. A tube was scored positive if undisturbed tubes showed visible loss of cellulose after three months incubation at room temperature in the dark. MPN calculations were taken from the tables of McKinney (1962).

Most Probable Number estimates were also done on subsamples of Nuphar leaves from litterbags buried in Wintergreen littoral sediments for one year. The leaf material was anaerobically transferred to nitrogen-flushed test tubes containing prereduced mineral salts. Stoppered tubes were vortexed for 10 minutes at high speed to dislodge colonizing bacteria and MPN series were set up from these tubes as described for the sediment series.

Laboratory Studies

Enrichment and Isolation of Cellulolytic Sediment Microorganisms

Both enrichment and direct isolation methods were used to obtained cellulolytic anaerobic microorganisms from Wintergreen Lake littoral zone sediments. Sediment enrichments were set up anaerobically by adding 1% (w/v) ball-milled filter paper, ground Nuphar, or cellulose purified from Nuphar to freshly collected sediments and incubating for 1 week to 3 months. Nuphar cellulose was obtained by acetic-nitric acid extraction of ground Nuphar (after Updegraff, 1969) followed by repeated hot and cold water washes of the residue. Cellulolytic MPN tubes were also used as sources for isolates. Enrichment cultures were streaked in the glove box onto anaerobic 1% carboxymethylcellulose (CMC) agar plates. After good growth was observed, isolated colonies were transferred to anaerobic 1% CMC broth. In addition to these enrichment procedures, ten-fold serial dilutions of freshly collected sediments were directly plated onto CMC agar plates and isolated colonies transferred to 1% CMC broth. Those isolates which liquified 1% CMC broth within 14 days were scored CMC-ase positive and retained for further study and characterization. CMC active isolates were considered pure cultures when microscopic and colony morphology appeared stable after three successive

streak platings on 1% CMC agar. The media composition of CMC broth or agar was: (per liter), Na_2HPO_4, 6.75 g; KH_2PO_4, 2.72 g; $NaHCO_2$, 0.5 g; NaCl, 0.40 g; $CaCl_2 \cdot 2H_2O$, 0.06 g; $MgSO_4 \cdot 7H_2O$, 0.095 g; $(NH_4)_2SO_4$, 0.228 g; yeast extract, 1.0 g; peptone, 1.0 g; carboxymethylcellulose (type 7MF, Hercules Inc., Wilmington, Del.), 1.0 g; 1% rezazurin, 2.0 ml; cysteine HCl hydrate, 0.5 g; agar (if used as solid media), 10 g. The relative CMC-ase activity of each isolate was assessed by measuring viscosity loss after 72 h of cultures incubated anaerobically in 1% CMC broth. Viscosimetry was done at 19°C using Fenske-Ostwald viscosimeters after the method of Bell et al. (1955).

Isolate Characterization

Anaerobic cellulolytic microorganisms isolated from Wintergreen Lake littoral sediments were characterized according to basic microbiological procedures, primarily those described in the Virginia Polytechnic Institute (VPI) Anaerobe Laboratory Manual (Holdeman et al., 1977). Except where noted, all characterization tests were set up using pre-reduced media and incubated in the anaerobic glove bag previously described. Incubation was at room temperature.

Cultures (24-48 h) of sediment isolates in 1% PY-CMC broth were used as inocula for the characterization tests (Table 2). The same cultures were examined microscopically for Gram reaction, morphology, motility and presence or absence of spores. If no spores were seen in a culture, spore production was assessed by inoculating the organism into 1% PY-starch broth and pasteurizing for 10 minutes at 80°C. Outgrowth after pasteurization was considered evidence for sporulation (Holdeman et al., 1977). The isolates were plated on 1% Py-CMC agar plates lacking cysteine and incubated aerobically to determine aerotolerance.

Table 2. Characterization tests for Wintergreen Lake littoral sediment anaerobic cellulolytic isolates.[1]

Media	Test
24-48 h culture in 1% PY-CMC broth	Microscopy: morphology, motility, Gram reaction, presence of spores
	CO_2 and H_2 production
1% PY-CMC agar without added cystein	Aerotolerance (aerobic incubation)
1% PYG broth	End product analysis--volatile and non-volatile fatty acids
1% PY-starch broth	Pasteurization for 10 min at 80°C Starch hydrolysis
SIM media	H_2S production
Chopped meat media	Indole production Catalase production Digestion
Gelatin	Hydrolysis
Nitrate media	Nitrate reduction
Carbohydrate fermentation: 1% PY + added carbohydrate	Final pH in: 0.5% arabinose, 0.5% esculin, 1.0% fructose, 1.0% glucose, 1.0% lactose, 1.0% maltose, 1.0% mannitol, 1.0% mannose, 1.0% raffinose, 1.0% rhamnose, 1.0% salicin, 1.0% starch, 1.0% sucrose, 0.5% trehalose, 1.0% xylose, 0.5% ribose, 0.5% melibiose
Milk media	Digestion and curd formation

[1]Except where noted in text, procedures and media for these tests were according to Holdeman et al. (1977).

Hydrogen and carbon dioxide production by isolates was determined gas chromatographically from headspace gas of isolates grown up in 1% PY-CMC broth under 100% nitrogen atmosphere. Gas analysis was done on a Carle Model 8500 Basic chromatograph equipped with a thermistor detector and a 1/8" x 6' coiled stainless steel molecular sieve 5A column (80/100 mesh) and a 1/8" x 6' Porapak Q column (80/100 mesh) with a series bypass valve.

Other tests performed on the isolates, included sulfide production, indole production, catalase presence, meat digestion, gelatin hydrolysis, nitrate reduction, range of sugars fermented, and analysis of fermentation products of the isolates in 1% PYG broth (done as outlined in Holdeman et al., 1977). Gas chromatography of short chain fatty acid end products of isolates in 1% PYG was done using a Varian Aerograph model 1400 with thermal conductivity detector and a 0.125" x 6' stainless steel column packed with 15% SP-1220-1% H_3PO_4 on Chromosorb W AW (100/120 mesh). Operating conditions were as follows: detector temperature, 185°C; injector, 185°C; column temperature, 130°C; helium flow rate was 25 ml/min.

Laboratory Studies of Cellulose Degradation

1. Pure cultures

Four of the most commonly isolated bacterial morphotypes from Wintergreen Lake were examined for cellulolytic activity on different cellulosic substrates. Three types of cellulose were chosen to represent different substrate qualities. Carboxymethylcellulose (Type 7MF, Hercules Inc., Wilmington, Del.) is a soluble cellulose derivative. Ball-milled Whatman No. 1 filter paper (a-cellulose) has a highly ordered crystalline structure, and ground Nuphar represents a complex, natural cellulosic

substrate in which the cellulose is closely associated with other cell wall constituents such as lignin and hemicellulose.

Several commonly used methods for measuring cellulase activity were found to be unsuitable for the purposes of this study. Estimation of reducing sugars released into growth media as a measure of cellulose hydrolysis is useful only if hydrolysis occurs much more rapidly than uptake by the organism being tested (Halliwell, 1963). Cellulolytic bacteria, in which the cellulase enzymes are generally cell-bound, do not release reducing sugars or their release does not correlate closely with cellulose breakdown (Dinsdale, et al, 1978). Measurement of cellulase in spent culture media (Weimer and Zeikus, 1977; Ng et al., 1977) similarly presupposes an extracellular enzyme or enzymes. A weight loss method based on determination of residual cellulose after incubation was chosen as the best method of assessing hydrolysis of the insoluble cellulose substrates by Wintergreen sediment isolates.

Forty-eight hour cultures in anaerobic 1% CMC broth were used as inocula for triplicate vessels containing basal media with an added cellulosic substrate. Serum bottles (125 ml) containing 100 ml of 1% ground Nuphar media were inoculated in the anaerobic chamber with 0.1 ml of the 48 hour cultures. A set of serum bottles containing 0.5% ball-milled filter paper were similarly inoculated. Five hundred ml Erlenmeyer flasks containing 300 ml each of CMC broth (1%) received 0.3 ml of inocula. Experimental vessels were stoppered and incubated with shaking at room temperature. Cellulolytic activity of isolates on these substrates was assessed at 0, 1, 2, 4, 8 and 15 day intervals. Sampling involved aseptic removal by syringe of 0.5 ml of well mixed culture from Nuphar and filter paper bottles at each sampling date. Samples were extracted

in acetic-nitric acid reagent and assayed for residual cellulose as already described. From CMC flasks, 6 ml samples were removed aseptically and substrate loss was estimated by viscometry using Ostwald-Fenske viscometers at 19°C according to the procedure of Bell et al. (1955).

2. Sediment microbial activity on cellulosic substrates

Surface sediments (0-6 cm) collected in January and June of 1978 were treated anaerobically and homogenized in an Erlenmeyer flask with a magnetic stirrer in the anaerobic glove box. Twenty-five ml of mud was added to triplicate sets of 60 ml serum bottles containing either 1% (w/v) ball-milled filter paper, 1% Nuphar-extracted cellulose (summer sediments only), or 2% ground Nuphar. An additional set of three bottles containing unamended sediments was included as a control. Fermentative activity was monitored in the bottles by measuring gas production weekly with a water wetted syringe. At the end of 3 months, the cellulolytic population was enumerated using the MPN method described previously. The residual cellulose in the sediments was measured after drying at 50°C as previously described.

RESULTS

Field Studies

Environmental Parameters

Temperature in the surface sediments of Wintergreen Lake littoral zone over the period of study is shown in Figure 3. The sediment temperatures ranged from 2.0°C in late January to 26.4°C in mid-August. There was little change in temperature with increasing depth in the sediments, with differences of less than 1°C from surface to 20 cm. A

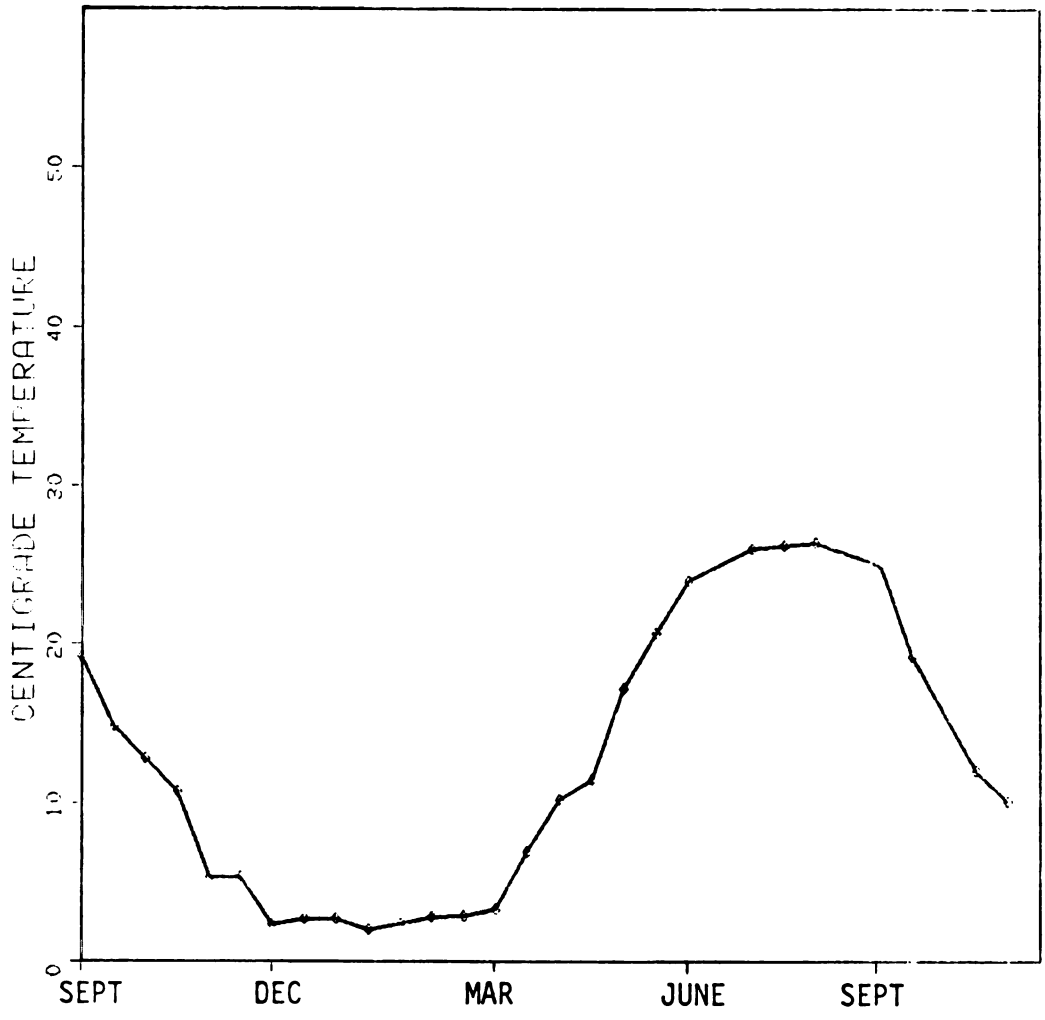


Figure 3. Temperature (^{o}C) in Wintergreen Lake littoral zone water column from 9/25/77 to 11/7/78.

rapid decline in temperature occurred between mid-September (20°C) and December (3°C). Temperatures ranged between 2-3°C during the period from December to March. Summer temperatures (May-September) ranged between 23-26°C.

Sulfide in interstitial water of surface sediments (mean values from 0-6 cm) reflected the reduced nature of the habitat (Figure 4). The range of sulfide values was 0.18 mg/l to 7.27 mg/l. During fall overturn (October-November, 1977) sulfide levels in surface sediments decreased sharply but never below 1 mg/l. The duration of these lowered sulfide levels was over two months, then sulfide levels increased rapidly to 6 mg/l after ice cover of the area. Sulfide reached its lowest concentration on 4/24/78, which corresponded to the spring overturn after ice-off. On this date, the surface sediments were almost completely free of sulfide. The interrupted anoxia was of brief duration; at the next sampling date (5/2/78) sulfide concentration was 2.87 mg/l. Sulfide generally increased throughout the summer, and reached its highest measured value (7.27 mg/l) in late August.

The cellulose content of the organic fraction of surface littoral sediments during the study period is shown in Figure 5. The values range from 0.99% to 2.21% of ash free dry weight. Highest values were measured during fall macrophyte senescence in 1977. Cellulose content declined in the sediments through the early fall, remained constant from December through May, followed by a further decline to its lowest measured value in mid-June. Cellulose increased again in the sediments in late summer and fall, reflecting input from macrophyte senescence, although fall values in 1978 never reached those measured during fall, 1977. The decreased cellulose pool size measured in 1978 relative to the previous

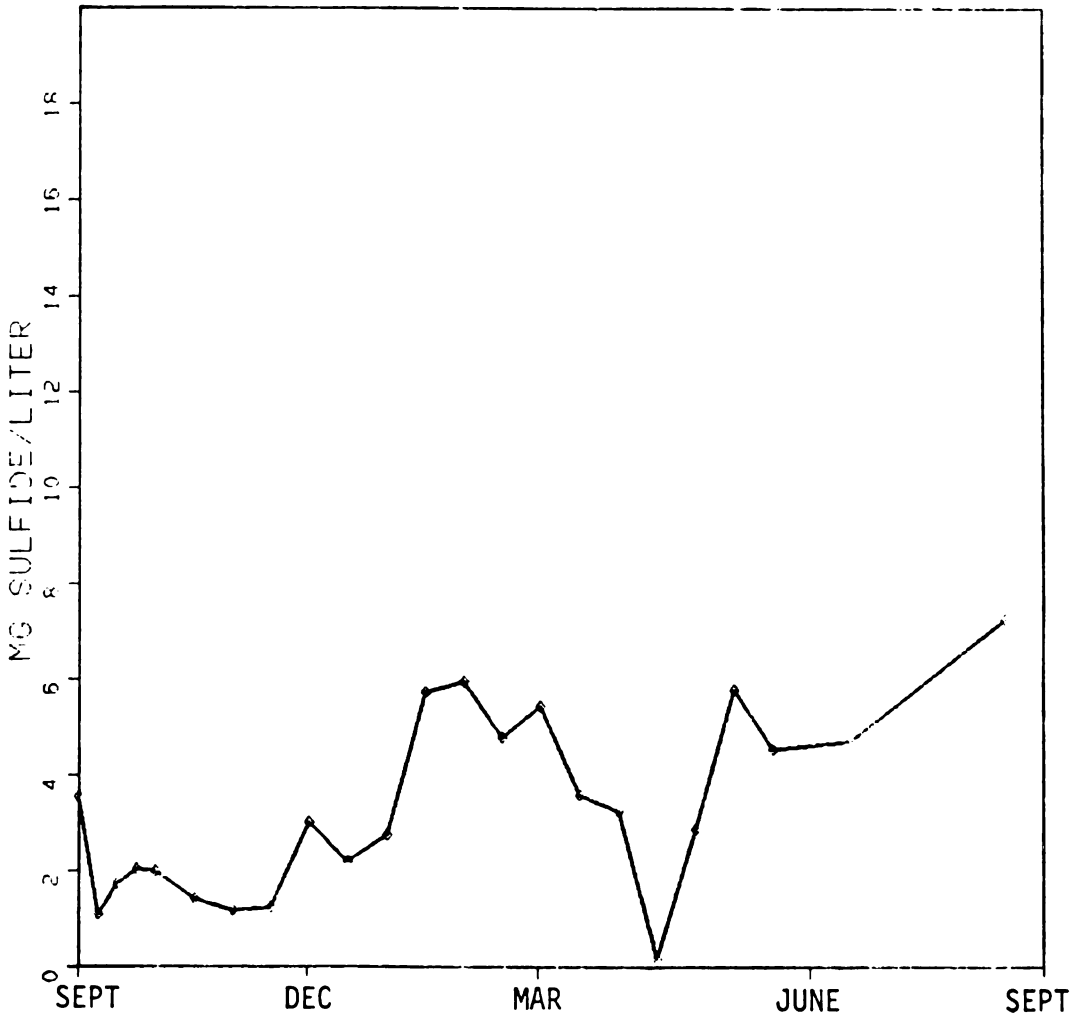


Figure 4. Sulfide concentration in surface sediments (0-6 cm) of Wintergreen Lake littoral zone from 9/25/77 to 11/7/78.

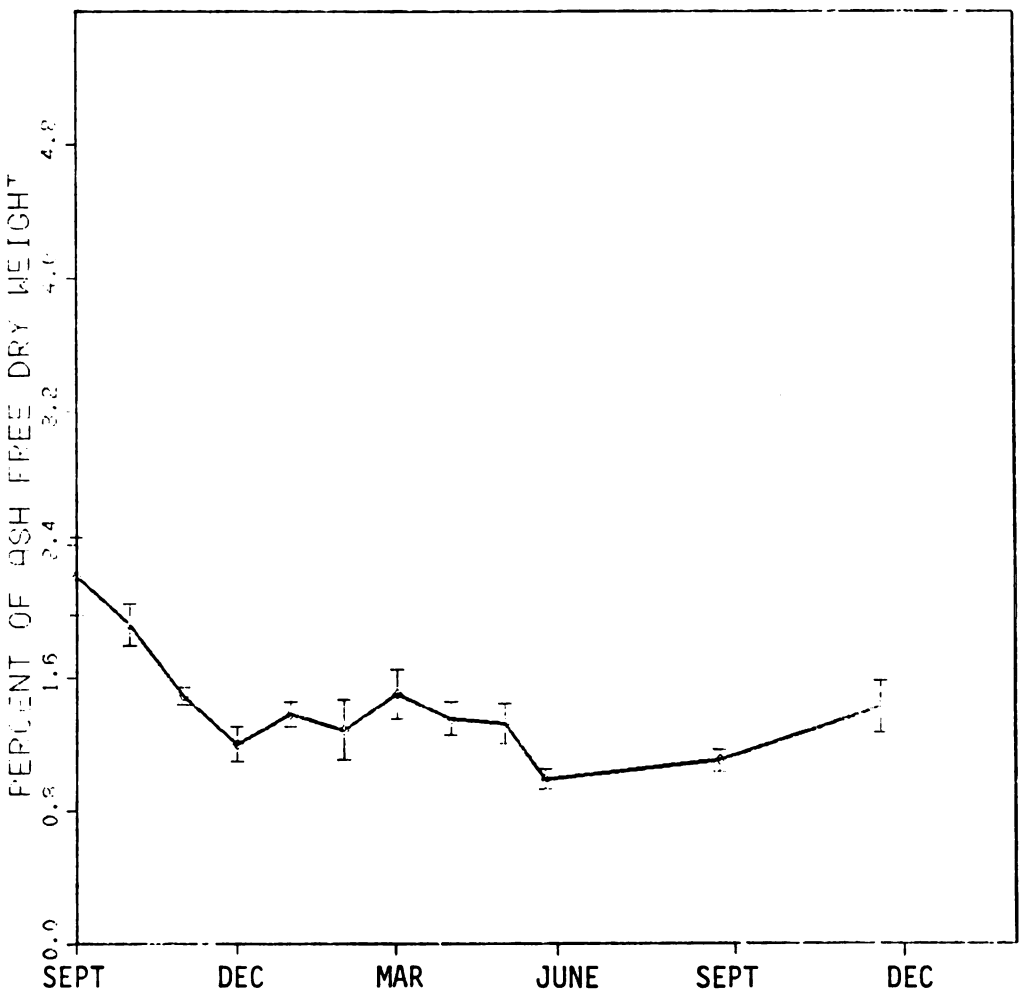


Figure 5. Pool size of cellulose in surface sediments (0-6 cm) of Wintergreen Lake littoral zone from 9/25/77 to 11/7/78, expressed as percent of ash free dry weight of sediment.

year correlates with the markedly decreased macrophyte production observed in 1978. A severe winter in 1977-78 resulted in the die-off of many Nuphar roots and rhizomes, which were found floating on the lake surface in the spring. This die-off probably accounts for the lower macrophyte production in 1978.

Litterbag Study

During autumn 1977, Nuphar senesced rapidly in Wintergreen Lake with the first cold weather in late September. The plant material fell to the sediments in a relatively intact state and was, in most cases, quickly buried in the flocculent sediments. Weight loss of Nuphar from litterbags buried in Wintergreen Lake littoral zone sediments over a one year period, represented in Figure 6, differed markedly for leaves and petioles. At the end of one year, total weight loss for leaf litterbags was 67.9%. For petiole litterbags no measurable material remained in the sediments after one year. Aside from absolute values, the weight loss curves for leaves and petioles were similar. There was an initial rapid weight loss during the first 8 weeks in the fall. Weight loss during the cold winter months was minimal followed by an increase in the summer. Some characteristics of the leaves and petioles were examined (Table 3) to explain the differences in decomposition rates. No marked differences in total fiber content, or in total lipid fraction were noted, but carbon/nitrogen ratio was almost twice as high in petioles as in leaves. Although lignin values were similar in both leaves and petioles the remaining two fiber components, cellulose and hemicellulose differed markedly. The density of the leaves is significantly higher than the petioles.

The cellulose loss in litterbag material with respect total weight loss for leaves and petioles was compared (Figures 7 and 8). In general,

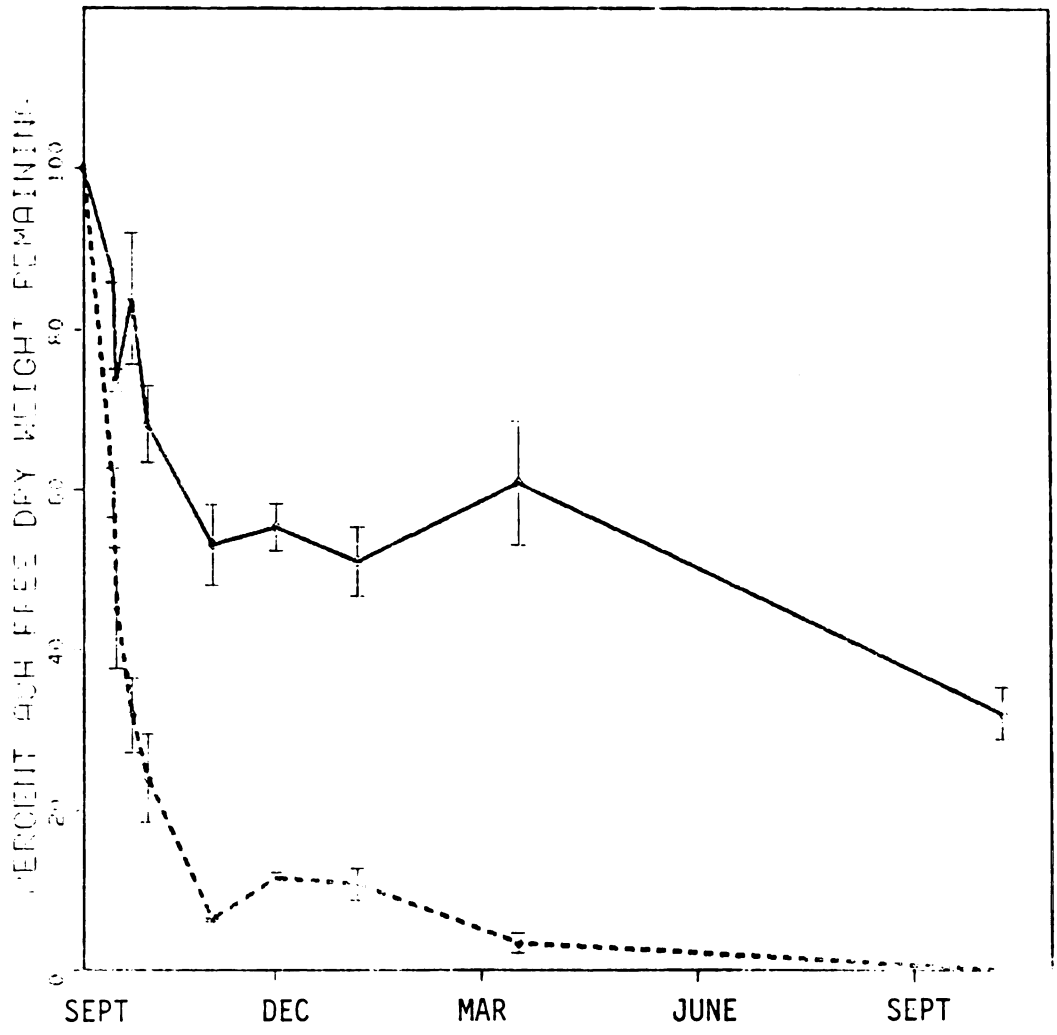


Figure 6. Weight loss of Nuphar leaves (solid line) and petioles (dashed line) from litterbags buried in littoral surface sediments (0-6 cm) of Wintergreen Lake from 9/25/77 to 11/7/78.

Table 3. General aspects of the composition of Nuphar leaves and petioles[1]

	Leaves[2]	Petioles[2]
% fiber	39.23 (0.48)	40.01 (0.07)
% hemicellulose	24.39 (0.03)	14.94 (0.07)
% cellulose	10.48 (0.03)	20.05 (0.08)
% lignin	4.39 (0.03)	5.11 (0.03)
chloroform/methanol extractable fraction (% lipid)	25.70 (nd)	23.97 (nd)
C/N ratio	11.24 (0.74)	20.51 (1.33)
bulk density, g/cc	0.206 (0.024)	0.043 (0.002)

[1] Fiber components were determined by the method of Goering and Van Soest (1970). Percent lipid was by chloroform/methanol extraction (Bligh and Dyer, 1959). C/N ratio was determined using a Carlo Erba Model 1104 elemental analyzer.

[2] Statistical error is expressed as one standard error.

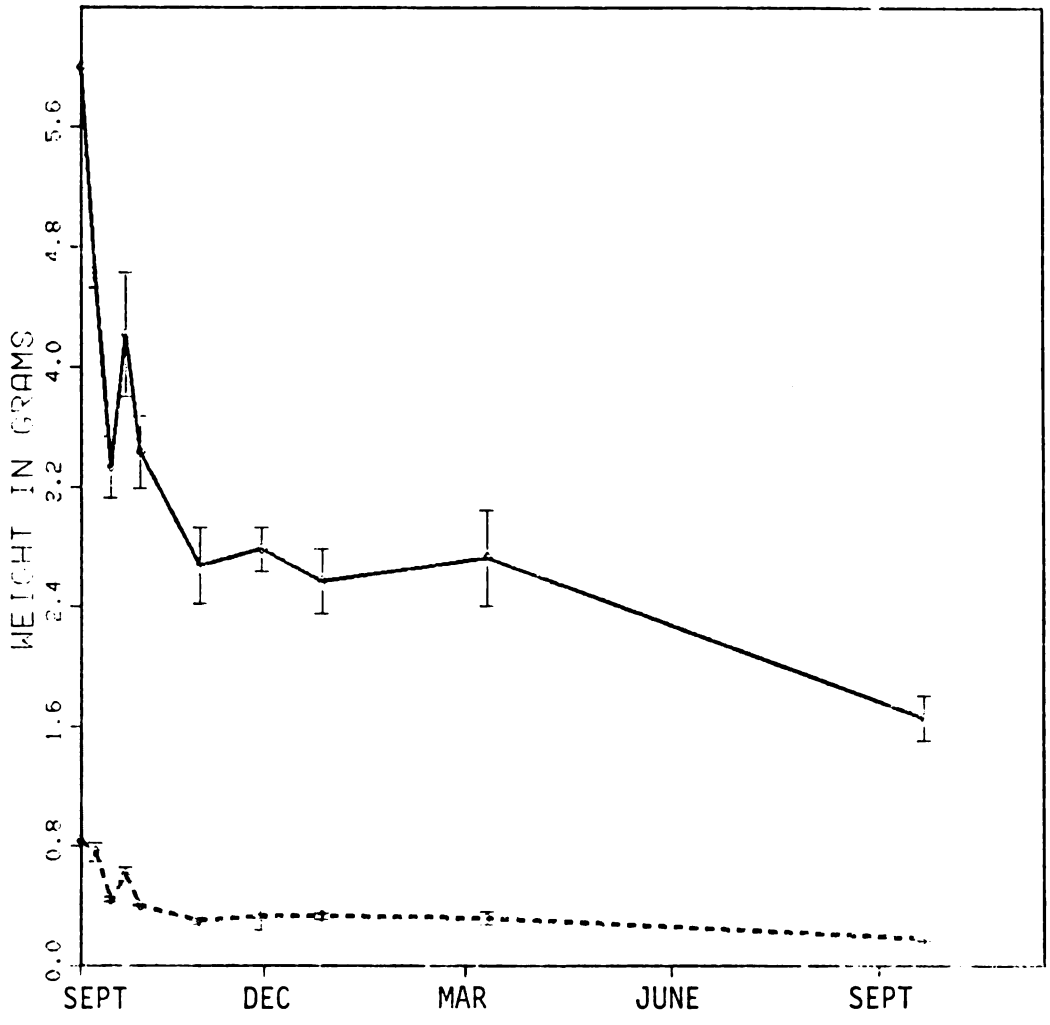


Figure 7. Total weight loss (solid line) and weight loss of the cellulose fraction (dashed line) of Nuphar leaf material from litterbags buried in littoral surface sediments (0-6 cm) of Wintergreen Lake from 9/25/77 to 11/7/78.

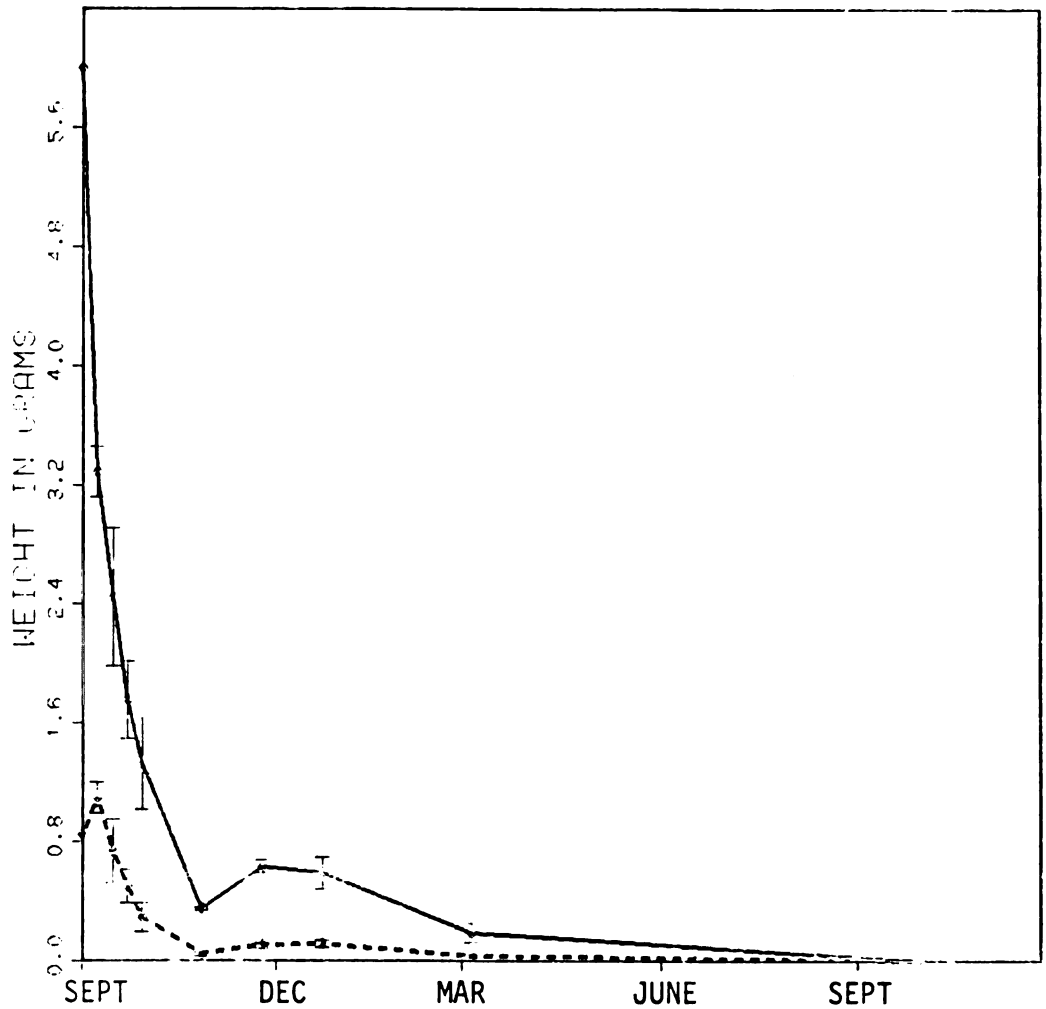


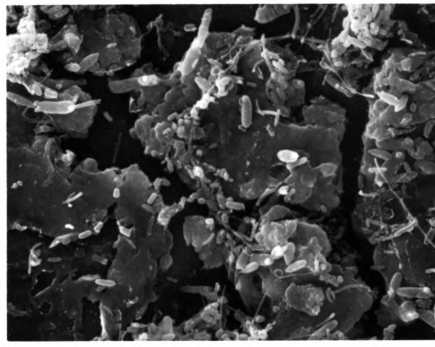
Figure 8. Total weight loss (solid line) and weight loss of the cellulose fraction (dashed line) of Nuphar petiole material from litterbags buried in littoral surface sediments (0-6 cm) of Wintergreen Lake from 9/25/77 to 11/7/78.

cellulose loss directly paralleled total weight loss. The cellulose fraction of leaf material incubated for one year in Wintergreen Lake littoral sediments decreased 78.9% while cellulose loss in petiole litterbags was complete at the end of the study period. In two cases, anomalous values were obtained in the first weeks of the study. First, the measured cellulose content of the petiole litterbags retrieved after a 1 week incubation in the sediments was higher than the initial content of the petiole material used in the study (Figure 8). In the second case, week 3 values for both total weight loss and cellulose loss in leaf litterbags (Figure 7) were lower than measured in week 2 samples.

Scanning electron micrographs of litterbag material included here showed differences in density and diversity of colonizing microbiota in early and late stages of sediment burial. Figures 9 and 10 are leaf material buried in Wintergreen Lake littoral sediments for one week and two weeks, respectively, during Fall 1977. Prior to burial, there was no epiphytic biota on upper leaf surfaces; the microbiota shown in these photomicrographs represent sediment microflora initially colonizing the leaf material. It was noted that the surface layer of the leaf began to erode after one week and was completely absent by week 3. Bacterial morphotypes colonizing 1 and 2 week old Nuphar leaf litter include various sized rods, cocci and spirochetes. Fungal hyphae were encountered only rarely during microscopy of litterbag material. Figure 11 represents leaf material buried in the sediments for 12 weeks. At that time the surface layer of the leaves was absent, leaving a relatively intact layer beneath. Bacterial density was lower and only 2 or 3 morphotypes colonized the leaf litter. Predominant among these bacteria were small rods (0.5 x 1.5 μm) and long rods (0.3 x 2-2.5 μm). These

Figure 9. Scanning electron micrograph of Nuphar leaf surface after burial in Wintergreen Lake littoral sediments for one week during fall 1977 (x 3900).

Figure 10. Scanning electron micrograph of Nuphar leaf surface after burial in Wintergreen Lake littoral sediments for 2 weeks during fall 1977 (x 2600).



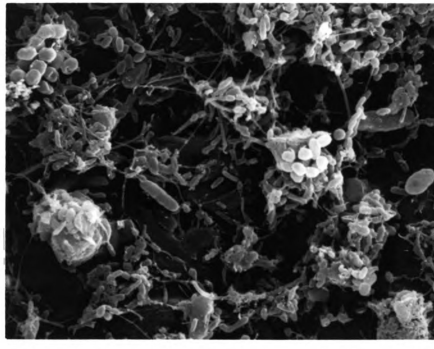
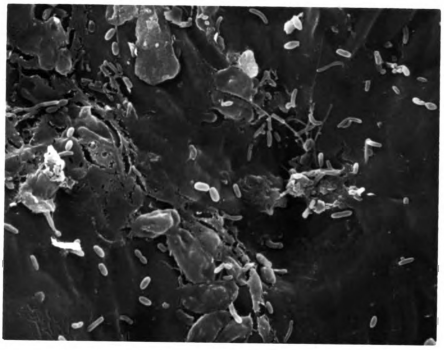


Figure 11. Scanning electron micrograph of Nuphar leaf surface after burial in Wintergreen Lake littoral sediments for 12 weeks during 1977-78 (x 3600).

Figure 12. Scanning electron micrograph of Nuphar leaf surface after burial in Wintergreen Lake littoral sediments for 27 weeks (x 2600).



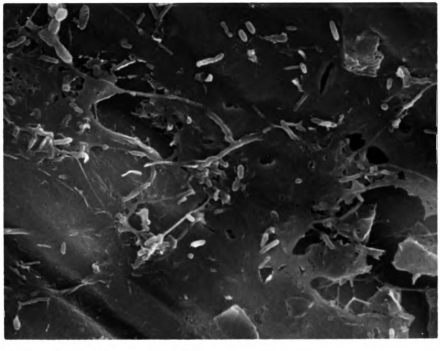
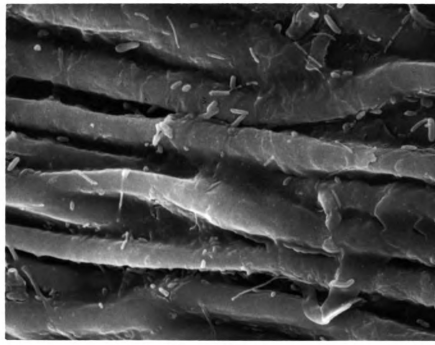
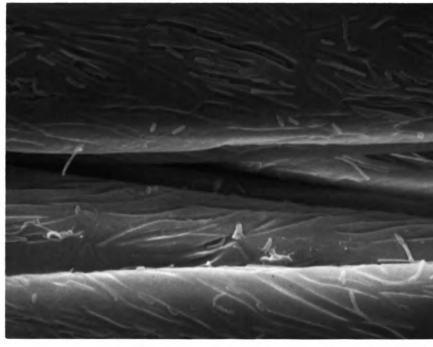


Figure 13. Scanning electron micrograph of Nuphar petioles surface after 27 weeks burial in Wintergreen Lake littoral sediments (x 2600).

Figure 14. Scanning electron micrograph of cotton fiber incubated in Wintergreen Lake littoral sediments for 1 week (x 3400).





two morphotypes persisted and were still evident at 27 weeks (Figure 12), where there was evidence of further breakdown of the plant tissue. Bacterial morphotypes seen colonizing leaf material at this stage are similar to those present on petiole material (Figure 13). Long, slender (0.3 x 2-3.5 μm) and occasional large rods (0.8 x 4-4.5 μm) are also seen.

Cotton Fiber Degradation

A profile of cellulolytic activity with depth in Wintergreen Lake littoral sediments is presented in Figure 15. Activity in the water column was very low with less than 3% tensile strength loss in cotton fiber at 12 cm above the sediment-water interface after 1 week's incubation in the lake. Activity increased slightly near the sediment water interface (+2 cm) but most activity occurred in the sediments. Greatest cellulolytic activity was measured at 2 cm below the sediment water interface. Cellulolytic activity gradually decreased with depth with the zone of greatest activity occurring between 0 and 6 cm depth.

A scanning electron micrograph representative of the incubated fiber (Figure 14) showed 3 distinct morphotypes of colonizing bacteria; a short rod (0.4 x 0.8-1 μm) an intermediate rod (0.4 x 2-3 μm), and a long rod (0.4 x 3.5-5 μm).

Enumeration of Anaerobic Cellulolytic Bacteria in Wintergreen Lake Littoral Sediments

Most Probable Number (MPN) estimates of anaerobic cellulolytic bacteria in the littoral sediments of Wintergreen Lake are given in Table 4. Seasonal variations in the cellulolytic bacterial density were small; the range between the lowest estimate (late winter, 1978) and the

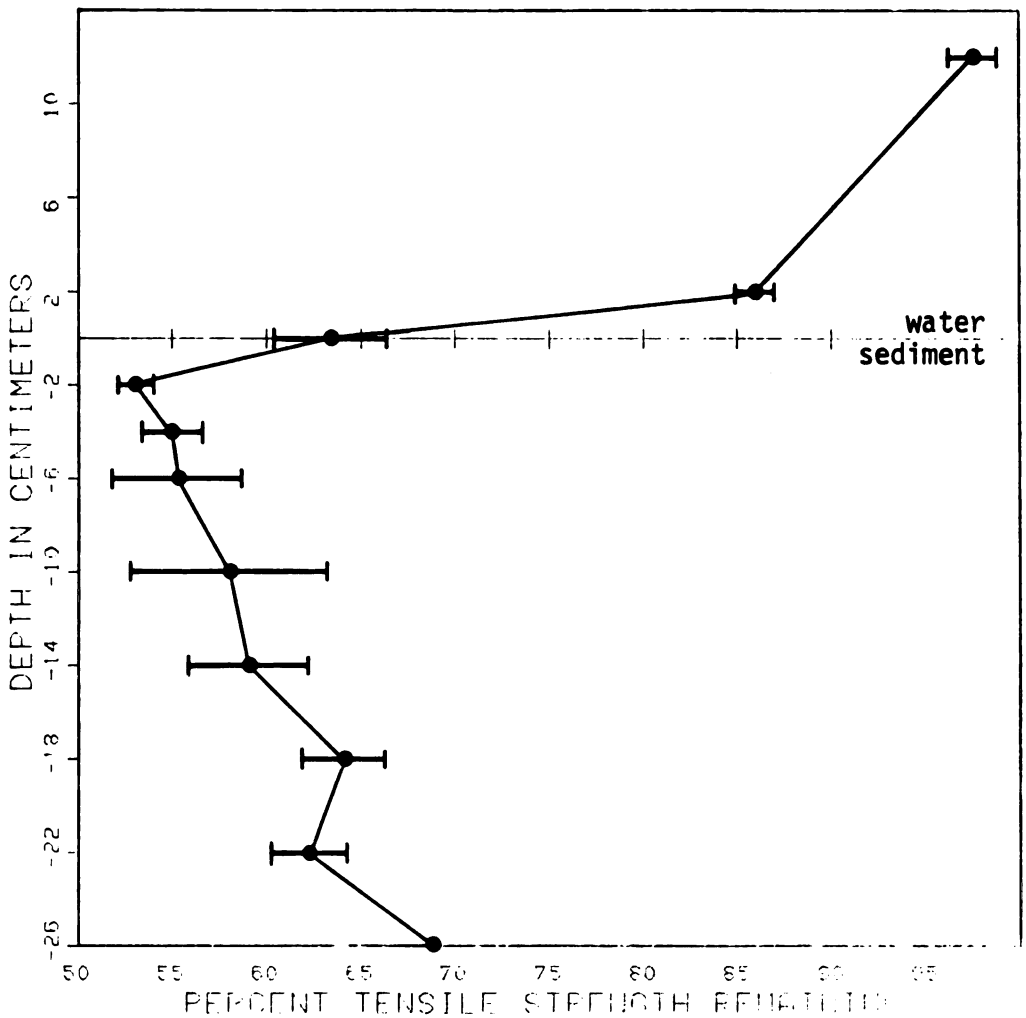


Figure 15. Cellulolytic activity with depth in Wintergreen Lake littoral sediments as measured by tensile strength loss of cotton fiber incubated for one week at various depths.

Table 4. Most Probable Number (MPN) determinations of anaerobic cellulolytic bacteria in Wintergreen Lake littoral sediments and colonizing year old Nuphar litterbag material.

Date	Source	MPN*	Mean
Late summer, 1977	sediment core, 0-6 cm	9.1×10^5	9.7×10^5
Late fall, 1977	sediment core, 0-6 cm	3.2×10^5	1.1×10^6
		1.8×10^6	
Late winter, 1978	sediment core, 0-6 cm	1.1×10^5	1.6×10^5
		2.1×10^5	
Late spring, 1978	sediment core, 0-6 cm	1.5×10^6	1.4×10^6
		9.0×10^5	
		1.8×10^6	
November, 1978	litterbag leaf material incubated for one year in Wintergreen Lake littoral sediments	8.6×10^7	9.7×10^7
		9.4×10^7	
		1.1×10^8	

*per gram of sediment or, for litterbag material, per gram of leaf material.

highest estimate (late spring, 1978) was less than one order of magnitude. Enumeration of the cellulolytic bacteria colonizing litterbag leaf material after one year in the sediments is also shown in Table 4. Most Probable Number values for colonization of this substrate are nearly two orders of magnitude greater than for the sediments.

Laboratory Studies

Isolation and Characterization of Cellulolytic Anaerobes

Sixty cellulolytic isolates were obtained from Wintergreen Lake littoral sediments during the course of the study. Isolates were divided into groups according to morphology and fatty acid end product analysis. Isolates from each group were then subjected to further biochemical tests (Tables 5, 6, 7). On the basis of these tests, a taxonomic affiliation has been suggested for each group, according to the VPI Anaerobe Manual (Holdeman et al., 1977) and Buchanan and Gibbons (1974). Table 8 gives the number of isolates represented by each group designation, their source of isolation and the relative CMC-ase activity of the group as a whole.

Group 1 contained the largest number of representatives (25) and was comprised of strictly anaerobic, Gram positive, non-sporing rods approximately 0.4 x 2-7 μm in size. Group 1 isolates produce mainly acetic acid in PYG broth, although some representatives form small amounts of lactic acid as well. Group 1 representatives were widely isolated by all three techniques used. Group 1 isolates were placed in the genus Eubacterium, and the species they most closely resembled was E. tenue (Holdeman et al., 1977).

Group 2 isolates (n = 15) differed from the other Wintergreen Lake isolates because of their facultatively anaerobic nature. The organisms

were motile, Gram positive rods, 0.3 x 1-2 μm in size. This group produced major amounts of lactic and acetic acids in PYG broth. Spores were not observed and no outgrowth occurred after pasteurization. Group 2 representatives were most often isolated by direct plating of fresh sediments onto 1% CMC media; however, isolates from this group were also obtained from both long term sediment enrichments and from MPN culture tubes. Tentative identification for the group was Cellulomonas flavigena.

Group 3 (n = 8) included strictly anaerobic, Gram negative rods, 0.3 x 2-4 μm in size. Acetic acid was the only VFA produced in PYG broth. Spores were not observed in Group 3 cultures and outgrowth did not occur after pasteurization. Group 3 isolates were obtained primarily from direct sediment plating but representatives were also isolated from long-term sediment enrichments. The suggested taxonomic affiliation for Group 3 was Clostridium clostridiiforme.

Group 4 was comprised of strictly anaerobic Gram positive sporeformers (n = 4), 0.4 x 2-7 μm in size. Only acetic acid was produced in PYG broth. Representatives of Group 4 were obtained by all 3 isolation methods used. Sporeforming capability and the strictly anaerobic nature of this group identified them as members of the genus Clostridium; the species they most closely resemble is C. sphenoides.

Group 5 isolates (n = 3) were Gram positive, strictly anaerobic rods (0.3 x 3-8 μm). In contrast with the other Wintergreen isolates, these organisms produced major amounts of propionic as well as acetic acid as fermentation products from PYG. Isolates in Group 5 were obtained from enrichment cultures and by direct isolation. The isolates from this group were identified as members of the genus Propionibacterium, most likely, P. acnes.

Table 5. General properties of bacteria isolated from Wintergreen Lake littoral sediments[1]

Group	Morphology	Gram Reaction	Relationship to Oxygen	Motility	Fermentation Products	Suggested Taxonomic Affiliation
1	Rod 0.5 x 2-7 μm	+	An	+	A(1)	*Eubacterium tenue*
2	Rod 0.3 x 1-2 μm	+	F	+	AL	*Cellulomonas flavigena*
3	Rod 0.3 x 2-4 μm	-	An	+	A	*Clostridium clostridiiforme*
4	Rod 0.4 x 2-7 μm	+	An	+	A	*Clostridium sphenoides*
5	Rod 0.3 x 3-8 μm	+	An	+	AP	*Propionibacterium acnes*
6	Rod 0.5 x 2-5 μm	+	An	-	ApB	*Clostridium indolus*
7	Rod 0.4 x 2-5 μm	+	An	-	AB1(iv)	*Eubacterium rectale*
8	Rod 0.3 x 2-4 μm	+	An	-	fAiBiV1	*Clostridium scatologenes*

[1](+), positive reaction; (-), negative reaction; (An), strict anaerobe; (F), facultative. Acidic fermentation products: (A), acetic; (L), lactic; (P), propionic; (B), butyric; (iB), isobutryic; (iV), isovaleric. Upper and lower case letters indicate major and minor amounts, respectively.

Table 6. Sugar fermentation reactions of isolated bacteria[1]

	Group							
	1	2	3	4	5	6	7	8
arabinose	-[a]	w	-	-		-	-	-
cellobiose	-[w]	w	v	-	a	-	-	-
esculin	w	a	w	w	-	w	w	w
esculin hydrolysis	+	+	+	+	+	-	+	-
fructose	a	a	a	a	w	a	a	a
glucose	a	a	a	a	a	a	a	a
lactose	-[a]	a[-]	-	-	-	w	w	-
maltose	-[a]	a	w	w	-	a	a	a
mannitol	-[a]	-	-	w	-	w	w	-
mannose	v	a	w[a]	w		w	w	w
melibiose				w		w		-
raffinose	-[a]	-	w[-]	a		w	a	-
rhamnose	v	-	w	w		w	w	-
ribose				w	-	w		w
salicin	w	-[a]	a[-]	a		w	a	-
starch hydrolysis	-	+	-[+]	-		-	-	-
sucrose	a[-]	a	w[a]	a	w	w	a	-
trehalose	w	-	w[-]	a	-	w	a	-
xylose	a	w	w	w		w	w	w

[1] (a), strong acid, pH 5.5 or below; (w), weak acid, pH 5.5-6.0 (for cultures in arabinose or xylose media, Ph 5.4-5.7 is considered a weak reaction, and pH below 5.4 strong acid); (-), acid not produced; symbols used as superscripts indicate reaction of 10-30% of strains.

Table 7. Biochemical reactions of isolated bacteria[1]

	Group							
	1	2	3	4	5	6	7	8
Gelatin liquifaction	w	-	w^-	-	+	w	-	-
Milk digestion				-		c		-
Meat digestion	-	-	d^-	-	-	d	d	-
Indole production	+	$+^-$	+	+	+	+	+	+
Nitrate reduction	-	-	-	-	-	-	-	-
Catalase production	+	+	-	-	+	-	-	+
H_2S production	$+^-$	-	$+^-$	+		-	+	-
H_2 production	$+^-$	-	$+^-$	+		+	+	+
CO_2 production	+	+	+	+	+	+	+	+
Pasteurization outgrowth	-	-	-	+	-	+	-	+
Spores observed	-	-	-	+	-	+	-	-

[1] (+), positive reaction; (-), negative reaction; (w), weak; (c), curd formation; (d), digestion; symbols used as superscripts indicate reaction of 10-30% of strains.

Table 8. Source and CMC-ase activity of isolates obtained from Wintergreen Lake littoral sediments.

Group	No. Isolates Represented	Source of Isolates			CMC Viscosity Loss after 72 h*	
		Direct Isolation	Enrichments	MPN Tubes	Mean Percent	Range Percent
1	25	9	9	7	57	100 - 20
2	15	12	1	2	57	80 - 20
3	8	6	2	0	57	80 - 20
4	4	2	1	1	50	80 - 20
5	3	1	2		60	80 - 20
6	2	1		1	65	80 - 50
7	2	1	1		70	80 - 60
8	1		1		20	---

* Cultures were incubated anaerobically in 1 percent CMC broth at room temperature.

Group 6 isolates (n = 2) were strictly anaerobic, Gram positive sporeformers which produced acetic, propionic and butyric acids in PYG broth. This group has been identified as a Clostridium species, resembling C. indolus. Isolates were obtained from MPN cultures and by direct isolation.

Group 7 included two strictly anaerobic, Gram positive rods, 0.4 x 2-5 μm in size. Acetic, butyric and lactic acids were produced in PYG broth. No spores were observed and outgrowth did not occur after pasteurization. This organism was isolated from enrichment cultures and direct sediment plating, and was identified as a Eubacterium species resembling E. rectale.

Group 8 consisted of a single isolate from an enrichment culture which degraded CMC rather slowly. The organism was a Gram positive, strictly anaerobic rod, 0.3 x 2-4 μm in size. Major amounts of acetic, butyric and isobutyric acids were formed in PYG broth, along with lesser amounts of formic and lactic acid. No spores were observed in culture, however, positive outgrowth after pasteurization suggested its affiliation with the genus Clostridium. It resembled the species C. scatologenes. Differentiation of the groups of clostridial species obtained from Wintergreen Lake was based primarily on their fermentation end products in PYG.

Cellulose Degradation by Wintergreen Lake Littoral Isolates

All three cellulose substrates were hydrolyzed by each of the Wintergreen littoral isolates tested (Table 9). Substrate loss for the different celluloses were calculated in terms of glucose equivalents (mg C_6 units hydrolyzed) for the purpose of direct comparison. In every case, CMC was hydrolyzed to the most extensively, followed by

Table 9. Cellulose degradation by Wintergreen littoral isolates under anaerobic conditions[a].

Group	Cellulose utilization[b], mg glucose equivalents/ml		
	CMC	α-cellulose	ground *Nuphar*
1	3.19 (0.02)	2.19 (0.01)	0.19 (0.002)
2	7.37 (0.03)	1.58 (0.01)	0.35 (0.002)
3	5.74 (0.02)	1.72 (0.02)	0.39 (0.002)
4	7.57 (0.01)	1.85 (0.02)	0.26 (0.002)

[a] incubation was at 21°C for 15 days under anaerobic conditions.

[b] substrate utilization was determined as residual cellulose for α-cellulose and ground *Nuphar* substrates, and as viscosity loss for CMC substrate. All values were converted to glucose equivalents for direct comparison.

α-cellulose and then the cellulose fraction of ground Nuphar. The Group 4 organism showed the greatest activity on CMC, approaching 100% substrate utilization by day 15. CMC hydrolysis by the Group 1 organism was slow during the first week of the experiment. By day 8, however, activity on this substrate had rapidly increased and final values for substrate utilization were higher on CMC than either of the other two cellulose substrates. Hydrolysis of α-cellulose by the isolates ranged from 21-68% of the same isolate's activity on CMC. Utilization of the cellulose fraction of ground Nuphar was significantly lower than that of the other substrates, ranging from 3-7% of CMC utilization during the same period. Although the final values for cellulose hydrolysis by Group 4 were similar to the other groups, this organism showed a lag in cellulolytic response to α-cellulose and Nuphar cellulose. For α-cellulose the lag was one day; for Nuphar, it was 4 days before active cellulolysis began. No lag was observed in CMC hydrolysis.

Decomposition of Cellulose by Sediment Microflora

A marked increase in fermentative activity measured as gas production in cellulose-enriched sediments was observed in both winter and summer sediments (Figures 16 and 17). Winter sediments sustained a higher control fermentation activity than did summer sediments, but enrichment with 1% α-cellulose produced a proportionately greater increase in activity in summer sediments than in winter sediments. This was also reflected by data on cellulose degradation by sediment microflora in cellulose-enriched and unamended sediments (Table 10). More cellulose was degraded on a percentage basis in unamended winter sediments than in unamended summer sediments, however, cellulose degradation in enriched

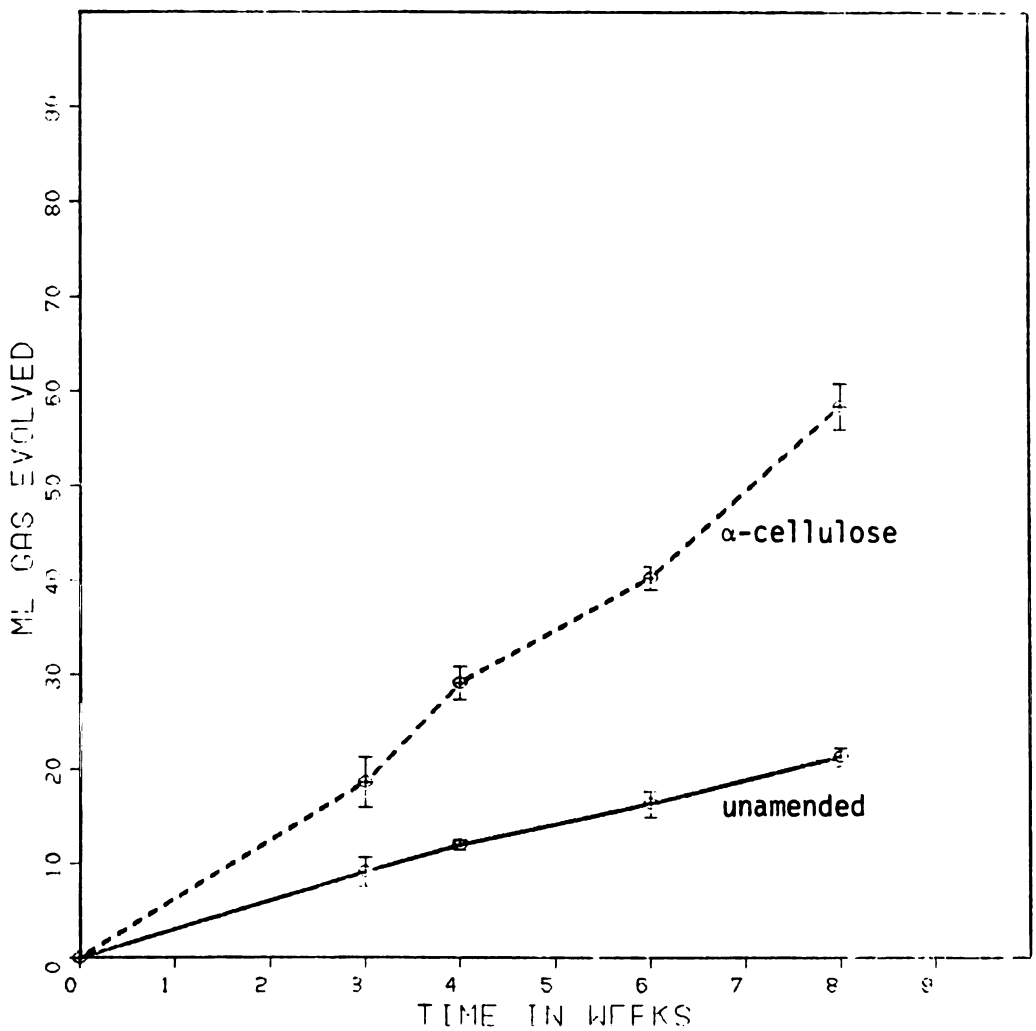


Figure 16. Fermentative activity in littoral sediments, Winter 1978, in response to cellulose enrichment. Dashed line represents sediments enriched with 1% α-cellulose; solid line is unamended sediment. Incubation was at room temperature under anaerobic conditions.

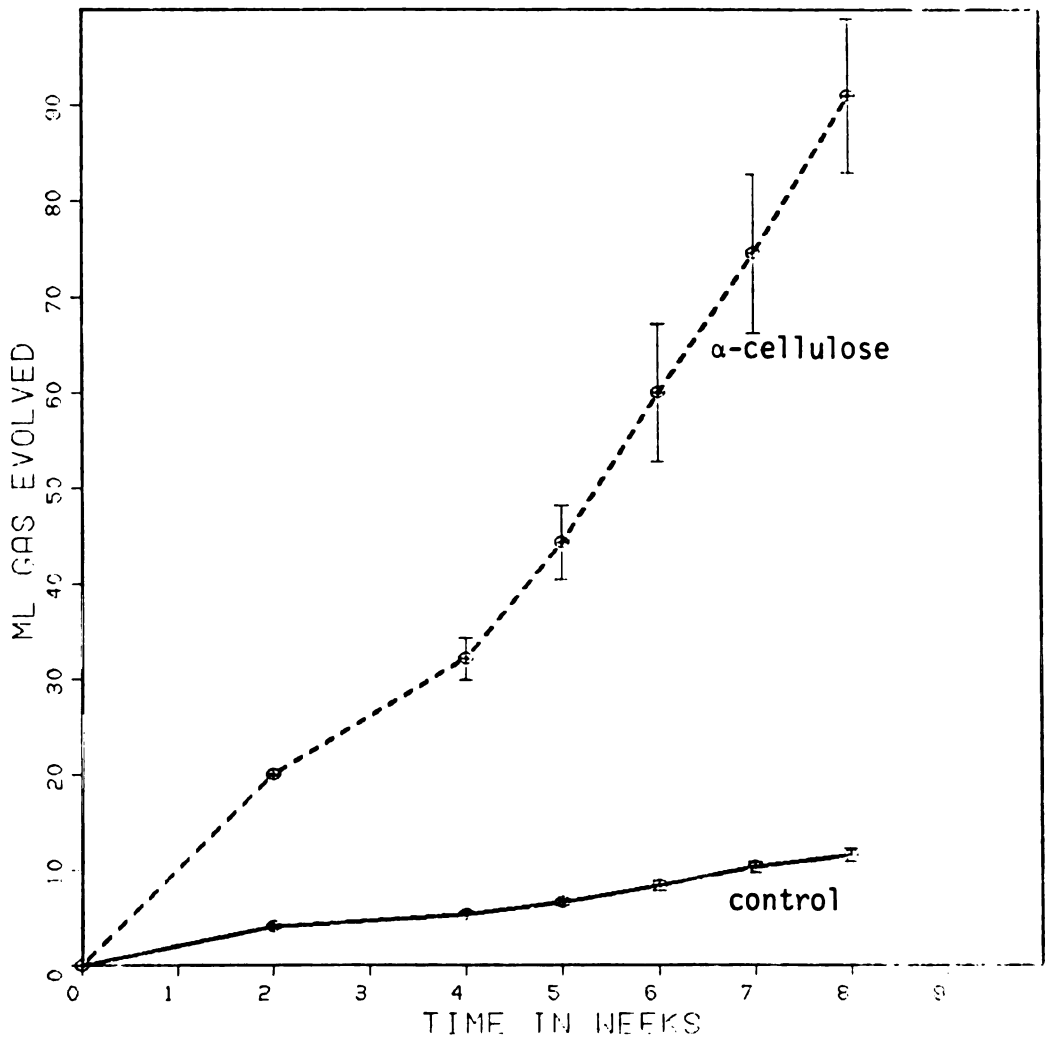


Figure 17. Fermentative activity in littoral sediments, Summer 1978, in response to cellulose enrichment. Dashed line represents sediments enriched with 1% α-cellulose; solid line is unamended sediment. Incubation was at room temperature under anaerobic conditions.

Table 10. Anaerobic degradation of cellulose in enriched and unamended Wintergreen Lake littoral sediments[a]

	Initial cellulose mg/ml mud	Final cellulose mg/ml mud	Cellulose degraded mg/ml mud	Percent degradation
Winter, 1978				
Unamended sediment	0.866 ± 0.010	0.550 ± 0.040	0.316	36.5
Sediment + 2% ground Nuphar	2.230 ± 0.046	1.113 ± 0.051	1.117	50.1
Sediment + 1% α-cellulose	10.866 ± 0.024	2.791 ± 0.064	8.075	74.3
Summer, 1978				
Unamended sediment	0.766 ± 0.033	0.646 ± 0.015	0.120	15.7
Sediment + 2% ground Nuphar	2.169 ± 0.021	0.785 ± 0.027	1.384	63.8
Sediment + 1% α-cellulose	10.780 ± 0.034	1.323 ± 0.053	9.460	87.7
Sediment + 1% Nuphar cellulose	7.742 ± 0.084	1.052 ± 0.084	6.690	86.4

[a] samples were incubated anaerobically at room temperature for 3 months as described in text.

sediments was somewhat greater in summer sediments. Cellulose degradation data for summer 1978 are also shown in Table 10 for sediments enriched with 2% ground Nuphar and with 1% cellulose purified from Nuphar. Degradation of the cellulose component of ground Nuphar is significantly lower than it was for α-cellulose in both winter and summer sediments. Relative to α-cellulose degradation, summer sediments showed greater activity on this substrate than winter sediments enrichments using cellulose extracted from Nuphar showed almost identical results as enrichments of α-cellulose from ball-milled filter paper.

Most Probable Number estimates of anaerobic cellulolytic bacteria in Wintergreen Lake littoral sediments before and after enrichment and incubation were compared in Table 11. There was a slight enrichment in bacterial numbers in unamended sediments during incubation, but numbers were distinctly higher in sediments enriched with either ground Nuphar or α-cellulose. In sediments with 1% α-cellulose added, numbers were 6×10^3 times higher than for unenriched sediments. MPN's from the ground Nuphar enrichments were only 15 times greater than unamended sediment values.

DISCUSSION

Physical Characteristics of the Littoral Sediments

The results of this study support the initial hypothesis that Wintergreen Lake littoral sediments provide a stable habitat for anaerobic cellulolytic activity. Interstitial sulfide concentrations reflect the relatively constant anoxic nature of the sediment environment. Due to the relationship between sulfide and redox potential, sulfide concentration

Table 11. Enumeration of anaerobic cellulolytic microorganisms in sediment enrichment experiment, Winter 1978.[1]

	MPN[2]
littoral sediments, January 1978	1.6×10^5
unamended sediment	1.1×10^6
sediment + 2% ground Nuphar	1.7×10^7
sediment + 1% α-cellulose	6.8×10^9

[1] MPN's were determined on sediments at day zero of the experiment, on the control and amended sediments after 90 days anaerobic incubation at room temperature.

[2] Cells/gram sediment

in interstitial water is an acceptable indicator of sediment anaerobiosis (Stumm and Morgan, 1970). Sulfide does not exist in lake or sediment systems under anaerobic conditions; it is rapidly oxidized to sulfate (Wetzel, 1975). Anaerobiosis in Wintergreen Lake littoral sediments is relatively constant with interruptions occurring only rarely and for brief periods (Figure 4). Although sulfide declined in surface sediments with the onset of fall overturn in 1977, values were never lower than 1 mg/l throughout the fall. This is also the period when organic matter inputs to the sediments are maximal due to macrophyte senescence. These inputs increase oxygen demand in the sediments leading to continued anoxia in all but the surface sediments. The rapid increase in sulfide accompanying ice cover demonstrates the strong tendency toward anaerobiosis of the sediments.

The low sulfide levels measured during spring overturn suggests that the sediments were oxidized for a brief period down to a depth of 6 cm. Recovery of anoxia in spring is slowed due to reduced oxygen demand since the organic pool in the sediments declines during fall and winter. Recovery of anaerobiosis occurred within two weeks, and increasing sulfide concentrations during spring and summer show that anoxia was undisturbed in the sediments for the remainder of the season.

The magnitude of aerobic metabolism of organic materials produced in Wintergreen Lake's littoral zone is unknown and is of interest in understanding the dynamics of total carbon turnover in the lake. Aerobic microbial activity can occur in the littoral zone in the water column and, to a certain extent, in the oxidized sediments which directly interface the oxygenated water column. Observations which

were made during the fall indicated that the majority of Nuphar leaves and petioles fell to the sediments upon senescence without much degradation in the water column. Once the plants reached the sediments, water column turbulence and the flocculent nature of the sediments caused rapid burial. Often, few plants were observed on the sediment surface within a week of senescence but many could be found by digging through the mud. Data obtained in this study indicate that Wintergreen Lake littoral sediments are primarily anaerobic in nature. Slow diffusion rates of oxygen through the sediments and the rapid utilization of this oxygen due to microbial metabolism function to severely compress the potential zone of aerobic activity to a band just a few millimeters deep at the sediment-water interface (Stumm and Morgan, 1970). Thus, although the relative magnitudes of aerobic and anaerobic decompositional activity have not been directly assessed, evidence indicates that anaerobic degradation of particulate organic material is more important in terms of macrophyte processing in Wintergreen littoral zone.

Another environmental factor which might influence the type of sediment metabolism is pH. Low pH due to buildup of acidic fermentation products has been shown to inhibit fermentation activity in anaerobic habitats (Brock, 1966). The high carbonate content of the sediments in Wintergreen Lake, however, acts as an effective pH buffer against potentially low pH which might occur due to fermentation activity and acid buildup; the pH of littoral sediment interstitial water ranges annually from 6.9 to 7.9.

Finally, the high sulfide concentrations present in Wintergreen Lake littoral sediments probably influence the sediment microbiota present. The effect of high sulfide levels in anaerobic habitats is not

well known. Sulfide does not build up appreciably in either the rumen or in anaerobic digesters, which are similar to the littoral sediments in other respects. Marine environments, in contrast, often build up high sulfide concentrations in the anaerobic sediments and high levels of biological activity are associated with these systems (Fenchel, 1971). Sulfide is known to be toxic to many organisms, however; it acts primarily to inhibit respiratory enzymes (Lehninger, 1975). This effect may not be important in the littoral zone sediments where anaerobic conditions limit the potential for respiratory metabolism.

Inputs to the Littoral Sediments

Cellulose forms significant portions of the aquatic macrophytes (over 30% of ash free dry weight in some species) which dominate the above ground biomass of the littoral zone (Godshalk, 1977). Most of this material reaches the littoral sediments and undergoes decomposition there. In addition some aquatic plants, like Nuphar, form extensive rhizome systems buried in the sediments which may represent up to 80% of the annual production of the plant (Wetzel, 1975). Although no studies have been made to quantitate input or turnover of this cellulosic material in the sediments, observations on the growth patterns of the plant show that degradation of rhizome tissue occurs continuously in the sediments at the end of the rhizome opposite the growing meristem. 50-75% of the measurable carbohydrates in littoral surface sediments are cellulosic; in contrast, insoluble carbohydrate polymers form only a small proportion (7%) of pelagial sediment carbohydrates. Instead sediment carbohydrates in pelagic areas are largely comprised of simple sugars and soluble polymers whose monomer composition reflects that of the blue-green algal populations which dominate in the pelagic water column (Salvas,

unpublished data).

In addition, anaerobic cellulolytic bacteria were not found among the heterotrophic populations of the Wintergreen pelagic sediments (Molongoski and Klug, 1976). These data point out the contrasting dynamics of carbon processing in littoral and pelagic regions of the lake, as well as the development of specialized sediment microbial populations in each habitat in response to the carbon input to the habitat (see also Molongoski, 1978). The cellulose-rich organic matter produced in the littoral zone is also decomposed primarily in the littoral zone of the lake; transport of this material to pelagic sediments, at least in Wintergreen Lake, appears not to be of great importance. Thus, cellulose is an important driving force in littoral sediment metabolism in Wintergreen Lake.

Degradation of Cellulose in Wintergreen Lake Littoral Sediments

The process of cellulose degradation in lake sediments is generally most active in a zone near the sediment surface. For Wintergreen Lake littoral sediments, cellulolytic activity is greatest a few centimeters below the sediment surface (Figure 15); the zone of highest activity occurs between 2 and 6 cm below the sediment-water interface. Similar results have been reported by Laurent (1969) over an annual cycle in a eutrophic pond in France, and by Fleischer and Larsson (1974) in Swedish lakes of differing trophic levels. In this surface zone, higher levels of utilizable substrate would be expected from recent organic inputs to the sediments.

The variations in cellulolytic activity occurring in Wintergreen littoral sediments are partly the result of fluctuations in annual temperature and the seasonal nature of organic matter inputs to the

sediments. Inputs due to fall macrophyte senescence result in high levels of cellulosic material in littoral zone sediments. This period is accompanied by declining temperatures which reach minimum values by late November. Total weight loss and cellulose weight loss from Nuphar litterbags is rapid during the initial stages in the fall, but slows significantly with the onset of winter temperatures from December through March. The cellulose pool size in Wintergreen littoral sediments shows a similar trend. Cellulose content measured in the sediments in September (2.2% of ash free dry weight) decreases steadily through the end of November and then remains constant (1.4% AFDW) until March. Microbial metabolism does not cease entirely in littoral sediments during the winter, as evidenced by microbial H_2S production and buildup in the sediments during the winter, but carbon turnover, at least of Nuphar litter, is slowed significantly during the cold winter months.

Most Probable Number values for cellulolytic anaerobes found in Wintergreen Lake are quite similar to the numbers estimated by Fjerdingstad and Berg (1973) in the sediments of eutrophic lakes in Denmark and Greenland; their estimates ranged from 0.6 to 8.9 x 10^6 organisms/gram of mud. Estimates of anaerobic cellulolytic bacteria in Wintergreen Lake littoral sediments follow seasonal temperature variation although numbers remain relatively high through the year (Table 4). Bacterial numbers increase slightly in late fall compared with the late summer (pre-senescence) estimate. This corresponds with increased cellulose levels in the sediments and occurs in spite of decreased sediment temperatures. The 10-fold lower counts in late winter correlate with the lower substrate levels and with the lower temperature. The increase in bacterial numbers in spring reflects a period of active

cellulose degradation in the sediments due to increased temperature. This activity resulted in the lowest cellulose pool size measured during the year in the sediments (0.99% AFDW).

Degradation patterns observed in the litterbags during the year, and the cellulolytic bacterial numbers in the sediments appear to be directly related to fluctuations in the pool size of cellulose and seasonal sediment temperatures. The use of bacterial numbers to estimate cellulolytic activity in the sediments is, however, speculative. The maintenance of a large community of cellulolytic bacteria during the winter period when litterbag weight loss and sediment pool size data indicate little degradation is interesting. The high winter numbers probably represent viable cells since sporeforming cellulose degraders were not isolated with great frequencey from the sediments. A possible explanation is that the metabolism of sediment cellulolytic bacteria slows in response to low environmental temperatures, while population density is, for the most part, maintained. Alternatively, low temperatures may limit the cellulolytic enzymes, thereby causing a switch in metabolism by the cellulolytic community to other substrates during periods of low environmental temperatures. Evidence for such temperature effects on metabolic activities has been noted in glucose metabolism of psychrotrophic pseudomonads (Lynch and Franklin, 1978).

Potential cellulolytic activity in littoral sediments is governed by temperature, substrate availability, and bacterial population size of cellulolytic organisms. Sediment response to cellulose enrichment differed because of these factors, in winter and summer sediments. In winter sediments, when the initial cellulose content of the sediments was relatively high, unamended sediments sustained a high fermentation

activity throughout the experiment. Although cellulose addition caused a significant increase in fermentation activity in winter sediments, the difference between gas production in enriched sediments and unamended sediments is only about one half the difference observed in summer sediments. The effect of enrichment on summer sediments was considerably greater overall, since gas production levels in unamended sediments were low. The relative response to enrichment of the winter sediments probably reflects the decreased initial cellulolytic population which would result in a slower response to enrichment. The greater population size of cellulolytic organisms in the summer sediments would make the reverse true. The higher initial cellulose content of winter sediments explains the high level of activity in unamended sediments compared to summer when low cellulose content of the sediments might prove limiting to the cellulolytic community.

It is apparent from this study and others that the quality, or susceptibility to microbial hydrolysis, of a cellulose substrate markedly affects its rate of degradation (Youatt, 1961; Norkrans, 1967; Cowling, 1975). Since cellulolysis occurs only when cellulase enzymes are in direct physical contact with a cellulose molecule (Cowling, 1975), any structural feature which interferes with direct contact between the enzymes and a cellulose substrate will decrease its susceptibility to hydrolysis. Cellulolytic fungi commonly produce extracellular cellulases which can diffuse into the complex structure of a cellulosic compound to effect hydrolysis; in contrast, most bacterial cellulases are cell-bound (Norkrans, 1967). This means that the cellulose substrates must be directly accessible to the bacterial cell before hydrolysis can occur. The physical proximity of cellulolytic rumen bacteria with their

substrates has been clearly shown in scanning electron micrographs (Akin, 1976; Latham et al., 1978; Dinsdale, 1978).

Cellulosic substrates range widely in their accessibility to hydrolytic enzymes. Physical and chemical factors which bear on the susceptibility of different celluloses to hydrolysis include the degree of crystallinity and the nature of the substances with which the cellulose is associated (Cowling, 1975).

The degree of crystallinity is an important factor controlling the susceptibility of a cellulose compound to hydrolysis. The molecular structure of cellulose consists of aggregations of cellulose polymers into microfibrils bound laterally to each other by hydrogen bonds, in varying degrees of order. Regions of highly ordered microfibrils are termed crystalline, whereas regions characterized by a less ordered structure are termed amorphous (Cowling, 1975). Cellulolytic enzymes readily hydrolyze amorphous regions of a cellulosic substrate but not the less accessible crystalline regions of the same substrate. Different celluloses may have very different degrees of crystallinity (Youatt, 1961). Carboxymethylcelluloses, for instance, have a low degree of crystallinity due to their substituent groups, and when the degree of substitution referring to the number of substituent groups attached to each glucose unit in the cellulose molecule) is between 0.5 and 0.7, these compounds are highly susceptible to cellulolysis. In contrast, cotton fiber is a highly crystalline cellulose which is much less susceptible to hydrolysis. Commonly used as a substrate for cellulase studies because it is almost pure cellulose in its native state, it is often subjected to chemical or physical treatments, such as ball-milling, to decrease its crystallinity, thereby increasing its accessibility to hydrolytic enzymes.

Substances associated with cellulose also affect its susceptibility to hydrolysis. Natural cellulosic compounds, i.e., the structural components of higher plants, are composed of varying combinations of cellulose, lignin and hemicellulose, often with minor amounts of protein, waxes, oils and other substances (Cowling, 1975). Highly lignified cellulose is very resistant to cellulolytic enzymes (Mandels et al., 1974; Polcin and Bezuchs, 1977). Cellulolysis does occur, however, if the lignocellulose is ground finely thereby making the cellulose component physically accessible to hydrolytic enzymes. Similar increases in cellulose hydrolysis have been noted in complex celluloses when the hemicelluloses associated with them were removed enzymatically (Youatt, 1961; Ghose and Bisaria, 1979). In the case of both lignin and hemicellulose, chemical linkages between the cellulose and associated substance have been proposed. Most work on the composition and structure of cellulose, however, indicates that the association is physical, not chemical, and that these non-cellulosic substances act as physical barriers to cellulose hydrolysis (Norkrans, 1967). In the same manner, waxy cuticles, produced by many plants to control water loss (Wilson et al., 1970) may form an effective barrier to cellulose hydrolysis. Finally, the low nitrogen and phosphorus content of most cellulosic materials has been observed to limit cellulose hydrolysis due, presumably, to the nutritional requirements of microorganisms for these elements (Cowling, 1975).

Enrichment of sediment with cellulose substrates provides evidence of the effect of quality on cellulolytic activity in Wintergreen Lake littoral sediments. Enrichment of both winter and summer sediments with ball-milled filter paper cellulose results in marked increases in

fermentation activity and in the population size of cellulolytic bacteria. This was despite the fact that the pool size of measurable cellulose in the unamended sediments at the beginning of both experiments was relatively high in each case compared to the amount of cellulose added (1-2 times greater). In both summer and winter sediments, ball-milled filter paper was more completely degraded than the cellulose fraction of ground Nuphar. In contrast, when Nuphar cellulose was extracted and purified before adding to sediments, the degradation response was almost identical to that for ball-milled filter paper. Hence, the cellulose in Nuphar is no less inherently desirable a substrate than is ball-milled filter paper to the cellulolytic microbiota; differences in decomposition are probably due to the structural and compositional complexities of the plant litter which act to slow cellulose decomposition of these natural inputs to the sediments.

The rate of decay of Nuphar and its cellulose component in situ declines with time, due probably to the declining quality of the substrate. That is, easily utilized portions of fresh litter are used at a high rate during early stages of decomposition, and the rate of decay slows when only more resistant portions of the substrate remain. These data fit a logistic curve, in which the decay coefficient decreases logarithmically with time. Some evidence of increasing refractility during decomposition of Nuphar is provided by the laboratory experiments of Godshalk (1977). While Nuphar degradation is more rapid at 25°C than at 10°C, total weight and cellulose weight loss curves are similar at both temperatures to results obtained in this study; i.e., an initial rapid loss, followed by a leveling off to a lower decomposition rate. Similar patterns have been reported for decomposition of Juncus in a

freshwater marsh (Boyd, 1971), and for hickory and maple leaves in an artificial stream (Wetzel and Manny, 1972). In other cases, significant initial losses (up to 20% of dry weight) during decomposition are attributed to physical leaching of soluble, labile materials (Howard-Williams and Davies, 1979; Mason and Bryant, 1975). While abiotic leaching can play a major role in organic weight loss during degradation (Harrison and Mann, 1975), the results of this study and those of Godshalk (1977) indicate that rates of decomposition of particulate organic matter such as cellulose can also follow logistic curves showing declining decay rates with declining quality of substrate.

Potential differences in substrate quality of natural inputs to Wintergreen littoral sediments are clearly demonstrated by comparing the extent of decomposition of Nuphar leaves and petioles (Figure 6). The possible explanations for the marked difference are not conclusive. A initial low carbon/nitrogen ratio (C/N) in a compound generally favors more rapid decomposition of an organic compound (Parnas, 1975). If C/N were a significant factor in controlling degradation in Nuphar, then the low C/N of the leaves (Table 3) should favor its degradation over that of the petioles. This is not the case. Lignin is low in Nuphar compared to other aquatic plants (Godshalk, 1977), and values for senescent leaves and petioles are similar, suggesting that this factor is not significant in explaining the differences between them. Total fiber content of leaves and petioles are very similar, but the leaves contain a greater proportion of hemicellulose and less cellulose than do petioles. Since hemicelluloses can mask cellulose from hydrolytic enzymes, this may partially explain the difference in degradation of the leaves and petioles. The percent composition of a given component such as lignin or hemicellulose in a

cellulosic compound, however, is not as important at the nature of its physical association with the cellulose so these observations are not conclusive. Lipid content does not differ markedly between leaves and petioles, and scanning electron microscopy of early stages of leaf decomposition in the sediments (Figures 9 and 10) indicates that no resistant cuticular layer occurs on the leaves which might prevent enzyme access to the cellulose.

The most likely explanation for differences in rates of leaf and petiole decay is a structural one, based on differences in their bulk density. The petioles of Nuphar are very spongy and porous, being used largely for gas transport between leaves and rhizomes (Sculthorpe, 1967). Dinsdale (1978) noted selective degradation of plant cell walls by rumen cellulolytic microorganisms in situ. He found that more accessible regions of the plant cells were hydrolyzed rapidly, but more closely packed regions were resistant to degradation. Similarly, the structure of Nuphar petioles makes them more accessible to cellulolytic enzymes and microorganisms than the very compact, non-porous structure of the leaves. In any case, degradation curves show that the quality of ellulose substrate varies between different substrates as well as within the same substrates in Wintergreen Lake littoral sediments.

Bacteria Associated with Anaerobic Cellulolytic Activity in the Sediments

The cellulolytic bacteria isolated from Wintergreen Lake littoral sediments are diverse;by both direct isolation and enrichment methods, 8 distinct groups of organisms which actively hydrolyze CMC have been identified. The majority of the isolates are strict anaerobes. The one facultative bacterium obtained was repeatedly isolated in both enrichment and direct isolations and is, thus, likely a resident member of the

sediment mocrobiota. The predominance of anaerobic cellulolytic bacteria in lake sediments has been noted by others (Laurent, 1969; Fjerdingstad and Berg, 1973) and is common in other anaerobic habitats such as the rumen (Hungate, 1975) and sewage sludge digesters (Maki, 1955).

Since most bacteria generally associated with the anaerobic decomposition of cellulose outside of the rumen are clostridial species (Skinner, 1960; Rheinheimer, 1974; Zdanowski, 1977), and Molongoski and Klug (1976) found that clostridia dominated the sediment microbiota of pelagial Wintergreen Lake, it is interesting to note the relative scarcity of cellulolytic sporeformers in littoral Wintergreen sediments. Although 25% of the cellulolytic bacteria isolated in this study were identified as members of the genus Clostridium, sporulating bacteria were rarely associated with Nuphar litterbag material and many of the clostridial isolates sporulated poorly or not at all in culture. Sporeforming ability is advantageous in unstable or fluctuating environments, such as soil (Brock, 1966). In the rumen, where a very stable anaerobic habitat is maintained, sporeforming cellulolytic bacteria are rarely encountered and are not generally quantitatively important in cellulose degradation (Hungate, 1966). In sewage sludge, the sporeformers and nonsporing rods isolated by Maki (1955) were so similar that he postulated that the nonsporing rods were clostridia which had lost their ability to sporulate. This characteristic may not be of selective advantage in Wintergreen Lake littoral sediments either, where substrate is continuously available and anaerobiosis is undisturbed most of the year.

Group 1 isolates are members of the genus Eubacterium, being nonsporeforming Gram-positive strictly anaerobic rods. They most closely resemble E. tenue (Holdeman et al., 1977). Cellulose hydrolysis has

been reported for one member of the genus, E. cellulosolvens, but the Wintergreen isolates differ from it by producing mainly acetate with some lactate from PYG. E. cellulosolvens characteristically produces butyric acid from PYG. The role of Group 1 isolates in sediment cellulose metabolism seems clear. They are easily isolated by all 3 methods used in the study, and morphotypes resembling Group 1 isolates colonize leaf litter and cotton fiber incubated in Wintergreen Lake littoral sediments. They are capable of cellulose hydrolysis of both ball-milled filter paper and the cellulose fraction of ground Nuphar, as well as the CMC substrate they were initially tested for hydrolytic activity on. On these criteria, Group 1 bacteria are presumed to be active members of the cellulolytic community in the sediments.

Group 2 isolates have been identified as Cellulomonas flavigena. A key feature of C. flavigena is its ability to hydrolyze cellulose, and most strains reported are capable of anaerobic growth (Keddie, 1974). Wintergreen strains were isolated primarily from direct isolations but were isolated 3 times from cellulose enrichment cultures and MPN cultures as well. These short rods resemble colonizing bacteria seen on leaf litter and cotton fibers incubated in the sediments. This indirect evidence, plus its frequency of isolation and the demonstrated ability of this organism to degrade all three cellulose substrates tested, including ground Nuphar, under anaerobic conditions, suggests that it has an important role in cellulolytic activity in anaerobic littoral sediments in Wintergreen Lake.

Group 3 isolates have been identified as members of the species Clostridium clostridiiforme (Holdeman et al., 1977) on the basis of their negative Gram stain, and production of only acetic acid from PYG.

They differ from Bacteroides species in that no major succinate is produced during glucose fermentation. Isolates from Wintergreen Lake were not induced to sporulate in culture and did not survive pasteurization. Holdeman et al. (1977) stated that identification of this species can be made without observing the presence of spores. Holdeman and Moore (1974) placed this organism in the genus Bacteroides as B. clostridiiformis on the basis of its tendency not to form spores, but it was moved recently to the genus Clostridium (Cato and Salmon, 1976). C. clostridiiforme is found as normal intestinal flora in birds, ruminant animals and man, so its presence in Wintergreen Lake littoral sediments probably reflects fecal inputs from resident birds or nearby farm waste runoff.

Cellulose degradation by strains of C. clostridiiforme has not been reported, although a number of species of Clostridium do have cellulolytic capabilities. C. thermocellum and C. thermocellulaseum are thermophilic cellulolytic clostridia which do not grow at temperatures below 35°C (Lee and Blackburn, 1974; Ng et al., 1977). C. cellobioparum is a mesophilic Clostridium which actively hydrolyzes cellulose (Smith and Hobbs, 1974; Holdeman et al., 1977). Group 3 isolates differ from C. cellobioparum in several key characteristics, i.e., Wintergreen isolates produce lactic acid as a fermentation product of glucose, and they to not sporulate. Group 3 isolates were obtained by all three isolation techniques and since the organism degraded all three cellulose substrates tested, including ground Nuphar, it probably plays an important role in cellulose degradation in the littoral sediments.

Sporeforming clostridia which hydrolyzed CMC were occasionally isolated from the study site. The group 4 organism, most prevalent of these, was a Gram-positive rod which produced only acetic acid and was

tentatively identified as C. sphenoides. Except for the formation of spores and the positive Gram-reaction, this group is very similar metabolically to C. clostridiiforme above, the key characteristic different between them being that C. sphenoides can ferment mannitol, while C. clostridiiforme cannot. No reports of cellulose fermentation by C. sphenoides strains have been found. Organisms in this group were isolated by both direct and indirect means and showed the ability in laboratory culture to degrade both purified and natural cellulose substrates, thereby suggesting for them a viable role in sediment cellulolytic activity.

Two other types of clostridia which actively hydrolyzed CMC were isolated from the study site. An organism resembling C. indolus was isolated from direct plating and from an MPN culture. This was the only group isolated from Wintergreen littoral sediments with strong proteolytic capabilities. The organism's activity on CMC was quite high; hydrolytic capabilities on other cellulosic substrates were not tested. These organisms probably do not play an important role in sediment cellulose degradation due to the rarity of isolation by diverse methods and their proteolytic nature.

One organism resembling C. scatologenes was also obtained from the Wintergreen study site. Its activity on CMC was low; other cellulose substrates were not tested. Presumably, its low activity on CMC and its rarity of isolation mean that this organism plays a minor role in cellulose breakdown in littoral sediments.

Two additional groups of strictly anaerobic bacteria were isolated occasionally from Wintergreen Lake littoral sediments, both non-spore-formers. A Gram-positive rod, similar to Eubacterium rectale was isolated by direct and enrichment methods. This organism hydrolyzed CMC rapidly;

other cellulose substrates were not tested. Cellulose degradation by E. rectale is not reported. The role this organism might play in Wintergreen Lake is unclear. It was rarely isolated but showed high activity in the hydrolysis of CMC. Until more information on its ability to hydrolyze complex cellulosic compounds is obtained, no conclusions can be made on its possible importance in Wintergreen Lake sediment metabolism.

The last group of bacteria isolated from the Wintergreen study site have been identified as members of the genus Propionibacterium, probably P. acnes. They are strictly anaerobic and were isolated from littoral sediments directly and from enrichment cultures. This species is the most common contaminant in anaerobic laboratory cultures (Moore and Holdeman, 1974), so the possibility exists that the isolates obtained in this study are laboratory contaminants. However, the presence of P. acnes in Wintergreen Lake sediments is not unlikely, since it is found as normal intestinal flora in birds, mammals and humans (Holdeman et al., 1977). Wintergreen Lake, a part of the Kellogg Bird Sanctuary, receives considerable input of fecal material from resident and migrating birds (Manny et al., 1975). No reports have been found of propionibacteria hydrolyzing cellulose. The Wintergreen isolates are active in CMC hydrolysis, but have not been tested on more complex cellulosic substrates. Until more is known about the cellulolytic capabilities of these isolates, no assessment of the role of this organism in sedimentary cellulose metabolism is possible.

Degradation of Cellulosic Substrates by Wintergreen Isolates

Estimates of the hydrolytic activity of sediment isolates on CMC and ball-milled filter paper can provide initial criteria for isolation. In addition, comparative data with other organisms is obtained, since

both are commonly used substrates in the study of cellulolytic microorganisms (Miller et al., 1960; Halliwell, 1963; Mandels et al., 1976; Latham and Wolin, 1977; Ng et al., 1977). It is, however, the hydrolytic ability of these organisms on the natural substrates available in the habitat which is crucial to determining their potential role in cellulose decomposition *in situ* (Mann, 1968). All of the Wintergreen isolates tested demonstrated activity on the cellulose fraction of ground *Nuphar* although to a considerably lesser extent than on alpha-cellulose or CMC. No great differences were noted in the extent of hydrolysis of cellulose from *Nuphar* among the species tested. *C. clostridiiforme* hydrolyzed *Nuphar* cellulose most extensively, utilizing 0.39 mg glucose equivalents/ml in 15 days. This is nearly twice as much as *E. tenue*, the slowest isolate, in the same period; *C. flavigena* and *C. sphenoides* fall within this range of hydrolysis values. Differences between the isolates in activity levels in either CMC or alpha-cellulose are also small. Thus, the affinity for and activity on each of the cellulosic substrates by the Wintergreen isolates is rather similar. Their collective response to different cellulose substrate types, however, is quite different. As discussed previously, these differences in rate of hydrolysis on the different substrates can be attributed to differences in the quality of the cellulose in the substrate.

One organism, *C. sphenoides*, did not show activity on alpha-cellulose or *Nuphar* cellulose until day 2 and 4, respectively, although the isolate's hydrolytic response to CMC was immediate. This suggests that part of the cellulase system, not functioning in CMC hydrolysis, is induced in this organism after a lag period by the presence of more structurally complex celluloses as substrate. Ng et al. (1977) found

similar inducible cellulases in strains of Clostridium thermocellum. The other 3 isolates showed no such lag in activity before hydrolyzing any of the substrates, which indicates that the enzyme systems in these organisms resemble the constitutive cellulase systems found in Cellulomonas (Beguin et al., 1977) and Clostridium thermocellulaseum (Hammerstrom et al., 1955).

Conclusion

Cellulose from the aquatic macrophytes which dominate the littoral zone of Wintergreen Lake provides a significant input to littoral sediments. High sulfide concentrations in these sediments during the study demonstrate their anaerobic nature. The localization of cellulolytic activity primarily in a zone just below the sediment surface, where fluctuations in anaerobiosis rarely occur, suggest that cellulose degradation is predominantly an anaerobic process in Wintergreen Lake littoral sediments. This is also supported by the observation that burial in littoral sediments is the most common fate of senescing plant material. Aerobic degradation, if significant, can only occur at certain times of the year (i.e., spring or fall turnover), or in a very compressed oxidized zone at the sediment surface.

Anaerobic processing of cellulose appears to be an effective mechanism for carbon turnover in Wintergreen Lake littoral sediments. On average, 84% of Nuphar biomass buried in anaerobic sediments was decomposed in an annual cycle. The pool size of cellulose in littoral sediments increases in the fall with plant senescence and declines thereafter, again demonstrating its turnover in the sediments.

The anaerobic cellulolytic bacteria found in Wintergreen littoral sediments are most often strictly anaerobic. Although sporeformers are

commonly found in other anaerobic systems (Maki, 1954; Skinner, 1960; Brock, 1966), they are probably not of great significance in this system. This is supported by microscopic observation of decaying litter, infrequency of isolation of sporeforming organisms, as well as the isolation of clostridial strains from the sediments which do not readily sporulate. Sporulation is commonly held to have survival value for microorganisms in fluctuating or unstable habitats (Brock, 1966). Hungate (1950) noted the lack or importance of sporeformers in the rumen and suggested that sporulating capabilities are no longer selected for in a stable habitat. It is possible that the habitat stability in Wintergreen Lake littoral sediments similarly reduces the survival value of sporulation in the cellulolytic community.

Wintergreen Lake has a high input of organic matter and fecal material from agricultural runoff and the large numbers of resident waterfowl (Manny, 1971). This provides a significant source of allochthonous bacterial seeding of the lake sediments. This constant input of bacteria and the stable anaerobic habitat provided by the littoral sediments encourages development of a diverse flora. Many of the cellulose degrading organisms isolated from the sediments are found as normal intestinal flora of birds and mammals. Except for Cellulomonas flavigena, the Wintergreen isolates have not previously been reported to hydrolyze cellulose, although cellulolytic members of the genera Eubacterium and Clostridium are known. Evidence for the important role of the most commonly isolated Wintergreen isolates in sediment cellulose decomposition was obtained by demonstrating their hydrolytic activity on natural cellulosic substrates.

Environmental temperature and the quality of cellulosic substrate

are additional factors which affect the rate and extent of cellulolytic activity in littoral sediments. Cellulolytic activity declines sharply during the winter due largely to temperature limitation, although cellulolytic bacterial numbers do not decrease dramatically in sediments at low temperatures. The winter decline in activity coincides with decreasing cellulose content of the sediments from autumn maxima through the winter, and very likely to the decline in quality of the cellulose remaining in the sediment pool.

The quality of the cellulosic substrate is an important limiting factor in cellulose degradation in sediments regardless of temperature or redox conditions (Godshalk, 1977). Both pure cultures of Wintergreen isolates and natural sediment cellulolytic communities demonstrated different hydrolytic activities depending on the quality of the substrate provided. Natural cellulosic substrates entering Wintergreen Lake littoral sediments include a range of qualities, as exemplified by differences in Nuphar leaf and petiole decomposition. Presumably, the quality range of input to littoral sediments extends even further, since there are other aquatic plants present in the lake which are more resistant to decay than Nuphar (Godshalk, 1977). Given this range of substrate quality, cellulose degradation in the littoral sediments will occur at different rates for different substrates. This pattern has potential value for a system in which input is largely seasonal, like the littoral sediments. Easily accessible cellulosic substrates can be utilized rapidly by the microbial community, while the more resistant celluloses form a slowly metabolized substrate reservoir which can maintain community stability during periods between input.

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